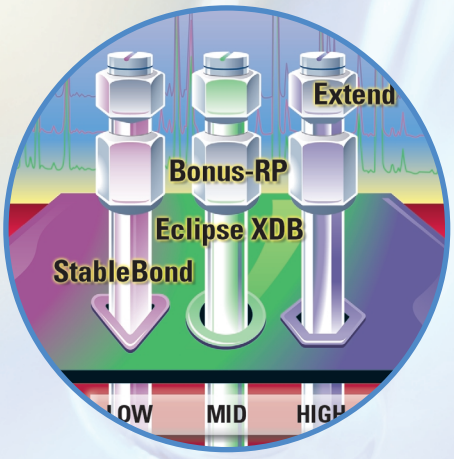
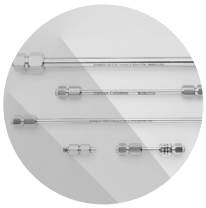
Agilent ZORBAX Column Selection Guide for Analytical HPLC

Resolution that Lasts



Agilent Technologies
Innovating the HP Way



Standard Fittings

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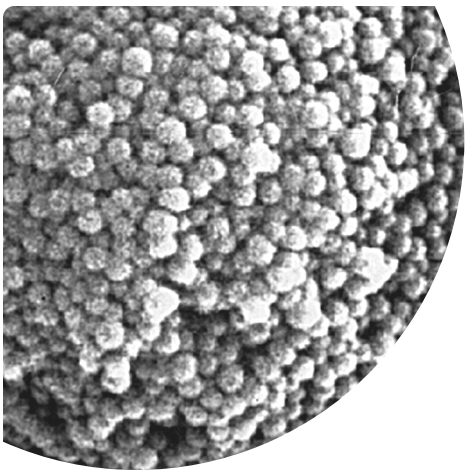
CombiHT

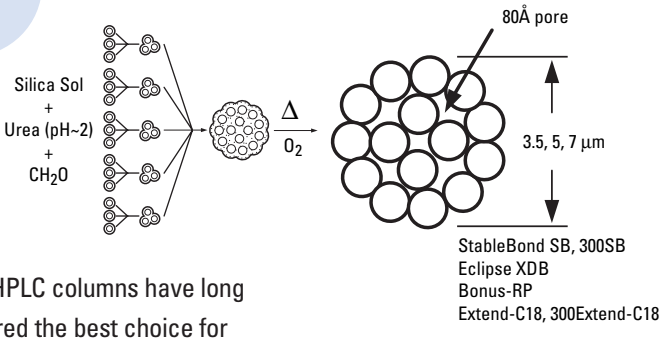


Agilent ZORBAX Silica is an Essential Part of Providing Resolution that Lasts

The ZORBAX Silica Manufacturing Process — The Making of a Rugged, High-Purity Silica

All Agilent ZORBAX columns are built from porous silica microspheres (PSM) based on silica sols. The silica particle is made of tiny, solid sol microparticles agglutinated in a patented polymerization process, then fused together at very high temperatures to form the final particle (Figure 1). These strong, durable silica particles are called ZORBAX Rx-SIL and are the base silica for most of the ZORBAX columns in this brochure.

FIGURE 1
Formation of ZORBAX Porous Silica Particles



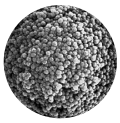
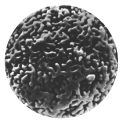
Silica-based HPLC columns have long been considered the best choice for maximizing resolution with long lifetimes and good column performance. The advantages of silica include high efficiency, small particle sizes, good mechanical strength and compatibility with a wide range of organic solvents. The quality and consistency of the silica support is critical. Therefore, close attention to the manufacture of the silica particles can result in improved chromatography for small basic molecules as well as peptides and proteins. Agilent Technologies manufactures silica particles using a unique process to provide a superior silica support for ZORBAX HPLC columns with total control over the manufacturing process.

The ZORBAX Rx-SIL process produces ultra-pure (99.995%) particles, with very low metal content. The final silica particle is fully hydroxylated and of low acidity. The Rx-SIL process also allows careful and reproducible control of pore size and particle size. These key features: purity (low acidity), strength, and careful control of pore size as well as particle size are critical to excellent chromatographic results and are the building blocks of superior ZORBAX bonded-phases.

Table 1 compares the process used to make the ZORBAX Rx-SIL particles to a second process — the Xerogel process — commonly used to make silica particles for HPLC columns. To produce silica with the key features that maximizes chromatographic performance — purity, strength, controlled pore size and particle size distribution, plus higher pH resistance — the Agilent ZORBAX process is an excellent choice.

Let's look at how these features improve your chromatography.

TABLE 1
Characteristics of ZORBAX Rx-SIL and a Contrasting Type of Silica

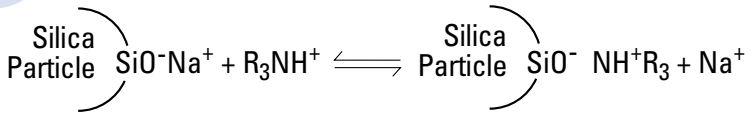
	ZORBAX Rx-SIL (Sol-type)	**Xerogel (SIL-type)**
		
Structure	Uniform Sub Particles	"Sponge-like", Polymeric Network
Purity	High (99.995%)	Low-High
Strength	High	Moderate
Pore Size, Particle Size Distribution	Narrow	Broad
Pore Size/Surface Area	80Å/180m^2/g	100Å/300m^2/g
Porosity (%)	50	70
High pH Resistance	Good	Poor

The Benefit of Silica Purity — Reduced Peak Tailing

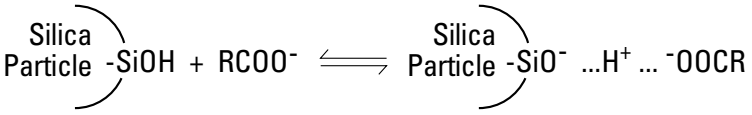
Peak tailing of basic compounds can be a major chromatographic problem. Peak tailing reduces chromatographic efficiency and the accuracy and reproducibility of results. The major cause of peak tailing is interactions between analytes and the silica surface (Figure 2). Typically the presence of acidic silanol sites on the silica surface cause this type of peak tailing. Trace metals in silica increase silanol acidity and peak asymmetry. These silanol interactions are reduced or eliminated by choosing a less acidic, ultrapure (99.995%) silica, such as ZORBAX Rx-SIL.

The improvement in chromatography is dramatic. Figure 3 shows the reduction in peak tailing using ZORBAX Rx-SIL versus a more acidic silica.

FIGURE 2
Potential Secondary Interactions with Silica Silanols and Ionizable Compounds



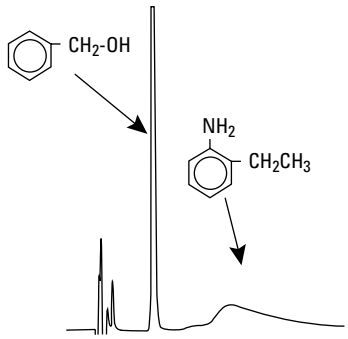
1. Ionized silanols (SiO^-) will ion-exchange with protonated bases (R_3NH^+) which can cause tailing and method variability.



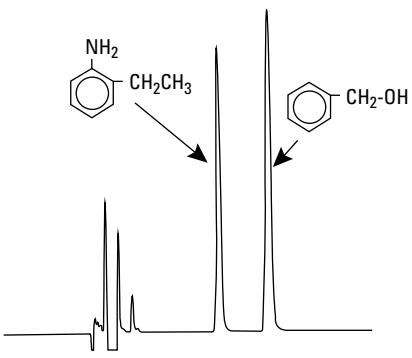
2. Unprotonated acids can compete for H^+ with protonated silanols.

FIGURE 3
Chromatographic Improvement Using Highly Purified ZORBAX Rx-SIL

Original ZORBAX SIL (1973)



Highly Purified ZORBAX Rx-SIL



Mobile Phase: 5% Propanol in Heptane
Flow Rate: 2.0 mL/min

The Benefits of Strong Particles — Greater Efficiency and Durability

During the silica manufacturing process, the agglutinated sol particles are sintered for increased strength. This improved mechanical stability allows ZORBAX columns to be packed at high pressures — up to 8000 psi! This results in a packed column with an exceptionally stable column bed that will not compress under normal or even high operating pressures — up to 5000 psi. This packed bed stability increases column lifetime using ZORBAX 3.5, 5 or 7 µm particles. When ZORBAX Rapid Resolution, 3.5 µm silica particles are used as the underlying support, high speed, high efficiency chromatography is possible without compromising column lifetime.

The Benefits of Careful Pore Size and Particle Size Control — High Efficiency and Better Reproducibility with More Column Choices

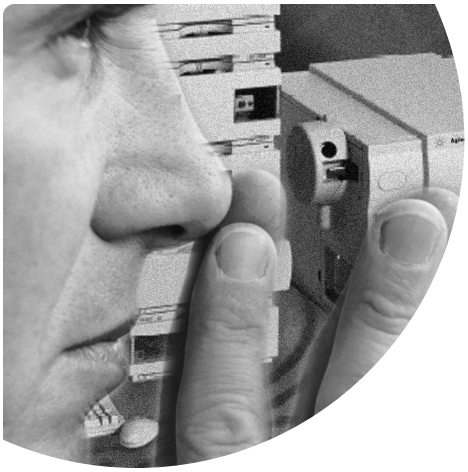
Accurate and closely monitored particle and pore size control for ZORBAX Rx-SIL produces reproducible retention behavior from column-to-column and lot-to-lot. The narrow, consistent particle size distribution of ZORBAX Rx-SIL particles maximizes efficiency and column bed stability. Column pressure is never unusually high due to "fines" - smaller particles at the low end of the particle size distribution.

Accurate and precise control of particle size allows specific 3.5, 5, and 7 µm ZORBAX Rx-SIL particles to be produced. The small 3.5 µm particle size is the basis for the Rapid Resolution, high-speed analysis columns designed to maximize resolution in shorter column lengths — ideal for LC/MS or any application demanding shorter analysis times. The 5 µm particles are an industry standard and provide high resolution in a wide variety of column dimensions. This particle size also provides high efficiency in a short preparative configuration — the CombiHT column — because careful particle size control means consistent pressure expectations within normal operating limits. The 7 µm particle provides the ideal balance between efficiency and operating pressure for longer preparative columns.

ZORBAX Rx-SIL — The Foundation for Many Bonded-Phases

With such strong performance characteristics, ZORBAX Rx-SIL particles have been developed into many effective bonded phases for solving key analytical problems. These include columns that can be used at extremes of pH, unmatched by any other silica-based columns. Because silica-based columns have different limitations at low and high pH, specific bonded-phase chemistries are required to provide longer column life over different pH ranges.

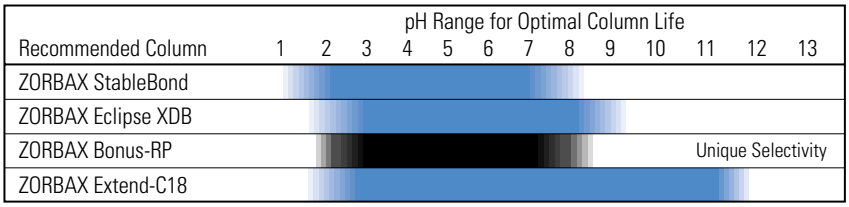
As a result, Agilent ZORBAX RP-HPLC bonded phases are designed to give extended column lifetime and reproducibility in the pH ranges that provide optimum and long lasting resolution, all starting with high performance ZORBAX Rx-SIL.



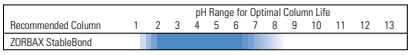
Agilent ZORBAX RP-HPLC Bonded Phases

Long Lifetime, Excellent Peak Shape, Superior Resolution from pH 1 - 12

Agilent ZORBAX reversed-phase bonded phases, based on Rx-SIL, provide the best peak shape and sample resolution, as well as the longest column lifetimes for the low, mid and high pH regions. The retention of ionizable compounds changes with pH, therefore achieving optimum resolution for complex samples requires establishing methods at any pH from pH 1 - 12. Several types of bonded phases are required to cover this wide pH range and provide maximum column lifetime. The ZORBAX columns described below use unique bonding chemistry to increase the lifetime of the bonded phase and the silica support where needed, thereby providing "Resolution that Lasts" at every pH.

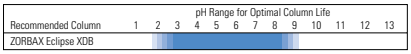

pH Range for Optimal Column Life
Recommended Column
1 2 3 4 5 6 7 8 9 10 11 12 13
ZORBAX StableBond
ZORBAX Eclipse XDB
ZORBAX Bonus-RP
Unique Selectivity
ZORBAX Extend-C18

For longest lifetime using low pH mobile phase — Agilent ZORBAX StableBond

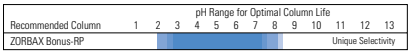


StableBond columns are made using bulky, patented, unique silanes that sterically protect the siloxane bond. Acid labile endcapping reagents are not used. The result is vastly improved column life and extraordinary chemical and temperature stability in the pH 1 - 6 range for a wide variety of phases (SB-C3, SB-CN, SB-Phenyl, SB-C8, and SB-C18). Excellent peak shape is expected for many basic compounds because of the ultra-pure Rx-SIL support.

For longest lifetime using mid-range pH mobile phase — Agilent ZORBAX Eclipse XDB or Bonus-RP

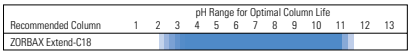


Eclipse XDB columns are best used in the pH range of 3 - 8 but can be used successfully over the extended pH range of 2 - 9. These columns provide excellent peak shape and high resolution for acidic, basic and neutral compounds. The densely-bonded, double-endcapped silica surface minimizes any silanol interactions and protects the silica support from dissolution.



Bonus-RP columns provide unique selectivity and applicability for mid pHs with an embedded amide linkage in a C14-alkyl chain. At pH 7 Bonus-RP provides excellent peak shape for even the most challenging basic compounds. Bonus-RP is protected from hydrolysis at low pH using the same sterically protecting bulky side chains as StableBond and also, is triple endcapped to minimize silanol interactions and improve stability at mid pH. Bonus-RP can be used without phase collapse in 100% aqueous mobile phases.

For longest lifetime using high pH mobile phase — Agilent ZORBAX Extend-C18



Extend-C18 columns are the newest addition to our product portfolio. These columns incorporate a unique, patented bidentate silane, combined with a double-endcapping process that protects the silica from dissolution at high pH — up to pH 11.5. Extend-C18 columns are best applied for separations of compounds that are either (1) basic and have little or no retention at low or intermediate pHs, (2) more stable or more soluble at high pH, or (3) basic and show poor peak shape at low or intermediate pH.

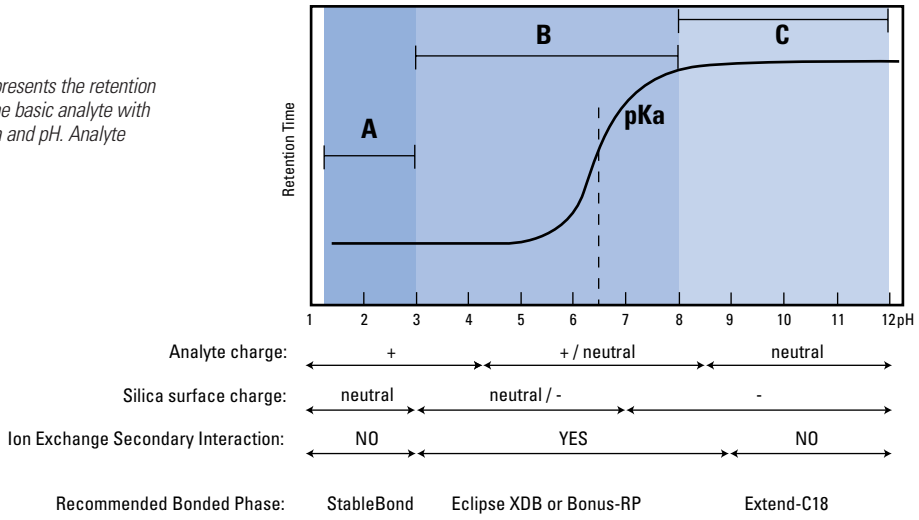
Method Development from pH 1 - 12

Chromatographic resolution between two or more peaks depends upon three factors — column efficiency, selectivity and retention. With ionizable analytes — bases and acids — all of these factors change dramatically with pH. For example, retention can be improved by changing the separation pH, so that analytes are separated in their non-ionized form. Changes in mobile phase pH also improve column efficiency because the ionization of the analyte and the residual silanols can both be altered. This minimizes secondary interactions between analytes and the silica surface that cause poor peak shape. Achieving optimum resolution requires changing the mobile phase pH. The following method development strategy explains how this is done with superior column lifetime.

Low, mid and high pH are the three general regions for chromatographic separations as defined in Figure 1. This figure highlights the benefits of performing separations of ionizable analytes in each pH region. Method development proceeds by investigating chromatographic separations first at low pH and then at higher pH until optimum results are achieved. The ideal column is available for each pH region.

FIGURE 1
Three pH Regions for HPLC Separations of Basic Compounds

This figure represents the retention behavior of one basic analyte with respect to pKa and pH. Analyte pKa is 6.5.



Low pH 1-3 — Region A

- Start method development at low pH, where silanols on a RP-HPLC column are protonated. This minimizes peak tailing by eliminating silanol/base interactions.
- At low pH basic compounds are positively charged, and their retention may be reduced.
- Acidic compounds may be protonated and have increased retention.
- Retention times are usually stable with small changes in pH, producing a robust method.
- Volatile mobile phase additives, such as formic acid or trifluoroacetic acid (TFA), are often used at low pH with LC/MS.

Mid pH 3 - 8 — Region B

- Develop methods at pH's at least 1 pH unit above or below the pKa to minimize changes in retention with small changes in pH .
- Some silica surface SiOH groups become SiO^- at pH 4 - 6; tailing interactions may be possible.
- Minimize interactions by selecting an endcapped column, using additives such as TEA (less desirable) or using "polar" bonded phases.
- Silica breakdown is prevented by innovative bonding chemistry, heavy endcapping and use of Rx-SIL.

High pH 8 - 12 — Region C

- In this region, basic compounds may be in their free base form.
- Increased retention and resolution of basic compounds are likely.
- Retention changes little in this region - thus robust methods can be developed.
- Silica breakdown is prevented by innovative bidentate column chemistry, heavy endcapping, use of Rx-SIL and optimum mobile phase.
- Ammonium hydroxide is an excellent volatile mobile phase modifier at high pH.

Method Development at Low pH is Preferred — StableBond Columns

StableBond — pH ≤ 3

With so many choices available, how do you select a column for method development? This is actually an easy decision. Most analytes today are acidic or basic - ionizable - and the best initial approach is to use a low pH mobile phase (Figure 1, Region A). Using a low pH mobile phase results in the best peak shape for basic compounds because ion-exchange interactions (BH^+ + SiOH ≠ secondary interaction) are eliminated. Many acidic compounds are non-charged, maximizing their retention at low pH. Therefore, StableBond columns are a good first choice providing the longest lifetime and most robust separations at low pH.

For standard analytical work, start method development with acetonitrile as the mobile phase organic modifier and 25 - 50 mM phosphate buffer (pH ≤ 3) as the aqueous component. This provides good pH control, necessary for the most reproducible analyses of ionizable compounds. StableBond SB-C8, or SB-C18 bonded-phases are usually chosen first because they effectively separate most compounds. Changes to the mobile phase (% organic, pH, ionic strength and organic modifier) and column temperature can be used to optimize the separation, as necessary. A unique feature of StableBond columns is the temperature stability. The temperature can be used as an additional tool to improve resolution.

StableBond Bonded-Phase Selectivity

The other StableBond phases — SB-CN, SB-C3, and SB-Phenyl — can be selected to improve sample resolution. All three of these bonded-phases are more polar than SB-C8 and SB-C18 and can change selectivity. Analysis time for non-polar compounds may also be reduced substantially while retention is maintained for polar compounds. In addition, the SB-CN bonded phase is an excellent choice when you have a mixture of polar and non-polar compounds. It will reduce the retention of the non-polar compounds and possibly eliminate the need for a gradient separation. All of these short-chain bonded phases provide the same exceptional stability as the SB-C8 and SB-C18 columns.

Method Development at Mid pH — Eclipse XDB and Bonus-RP Columns

Eclipse XDB — pH 7

If low pH is not desirable or acceptable for your separation due to poor analyte retention, solubility, selectivity or stability, then a mid-range pH, such as pH 7, should be investigated (Figure 1, Region B). This usually maximizes differences in retention and selectivity in comparison to low pH. The Eclipse XDB-C18 and C8 bonded-phases are normally the first choice for use at this pH because they provide high resolution and superior column lifetime. Phosphate buffer is usually the first choice for mobile phase modifier at pH 7 because its buffer range is pH 6.2 - 8.2. Phosphate buffers, although commonly used, should be used at temperatures lower than 40°C for maximum column life. A second choice for mid pH is acetate buffer since it buffers from pH 3.8 - 5.8 and is normally selected for LC/MS compatibility. Method development optimization can continue from here by changing the mobile phase, pH, and column temperature (up to 60°C) or by changing the bonded phase to Eclipse XDB-Phenyl.

Bonus-RP — pH 7

For even more dramatic changes in selectivity or to improve the peak symmetry of basic compounds in the mid-pH range, try the Bonus-RP column. The Bonus-RP column exhibits a modified reversed-phase behavior. When you choose this column, the method development process will be the same as with the Eclipse XDB columns. You can select the same types of buffered mobile phases and use the same organic modifiers; you can even vary the temperature up to 60°C. In general, the Bonus-RP is slightly less retentive than alkyl C8 columns because of the polar amine group embedded in the bonded phase. The Bonus-RP column has enhanced stability down to pH 2, so it can also be selected to improve peak shape of difficult basic compounds that tail badly on many other alkyl type phases. Bonus-RP columns can be used with 100% aqueous mobile phases.

Method Development at High pH

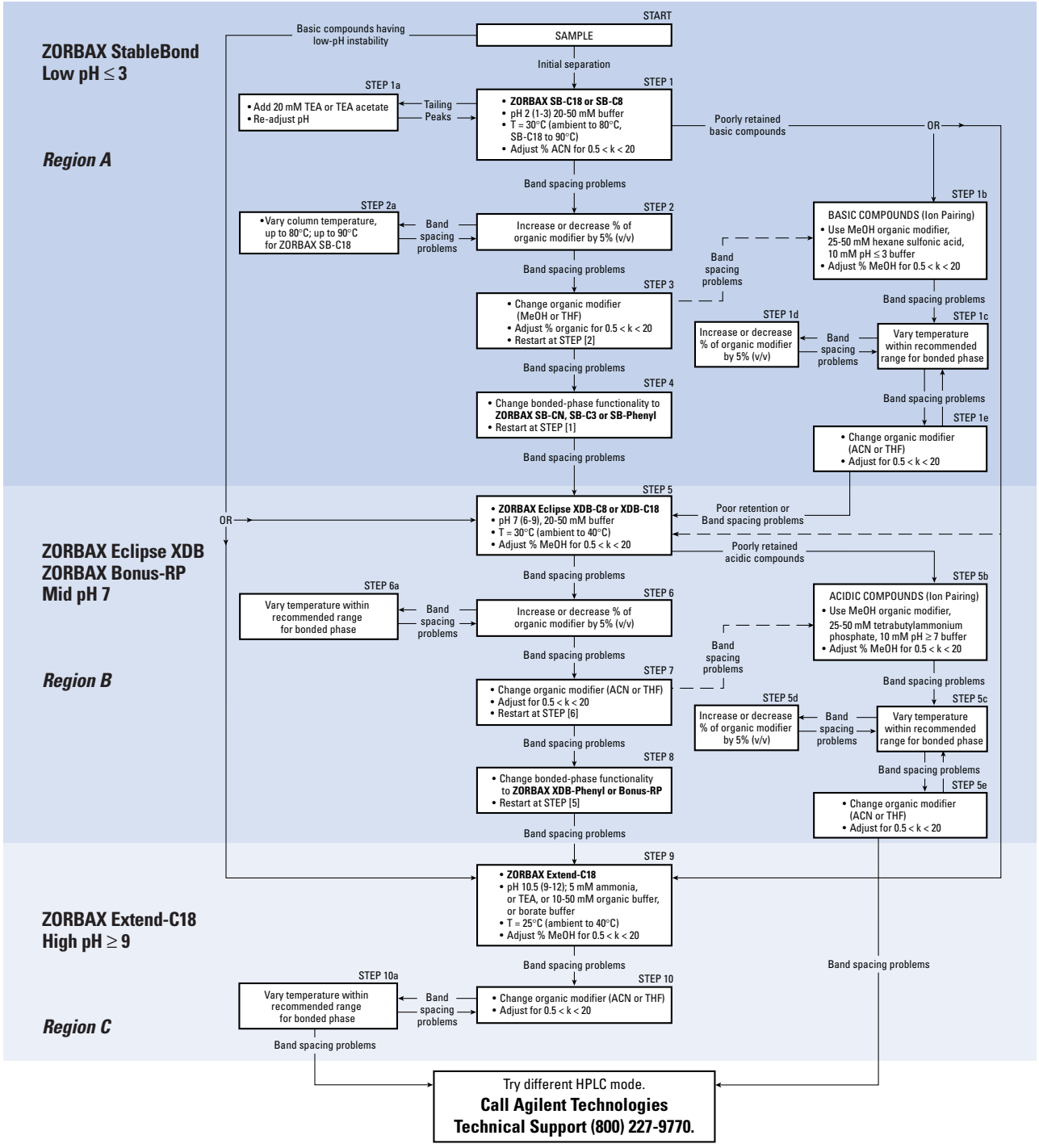
Extend-C18 Columns

At low or mid pH some separations of basic compounds may still not have enough retention or the desired selectivity. For these samples high pH separations may be appropriate (Figure 1, Region C). At high pH, it may be possible to separate bases in their free base form, where they are not charged. Retention increases and the chance of obtaining the desired selectivity improves. The Extend-C18 column performs well with high pH mobile phases — up to pH 11.5. For many basic compounds with a pKa of 9 - 10, this extended pH range allows the separation to take place at least 1 pH unit above the analyte pKa, where it will be in free base form. Method development using the Extend-C18 column is the same as on other columns — choose a buffered mobile phase appropriate for the pH range needed and an organic modifier. However, when using the Extend-C18 column organic buffers such as triethylamine, ammonium hydroxide and pyrrolidine are needed for maximum column lifetime; phosphate buffer is not recommended. Methanol is the preferred organic modifier.

ZORBAX Columns Provide Resolution that Lasts

ZORBAX columns have been carefully designed for optimum sample resolution and longest column lifetimes for the low, mid, and high pH ranges. Following the strategy described below simplifies method development using ZORBAX columns. You never have to take a column and make it work — your sample and mobile phases define the best column — so you can always achieve "Resolution that Lasts".

A Method Development Strategy that Optimizes Resolution and Column Life

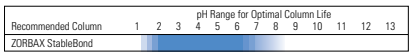

ZORBAX StableBond
Low pH ≤ 3
Region A
ZORBAX Eclipse XDB
ZORBAX Bonus-RP
Mid pH 7
Region B
ZORBAX Extend-C18
High pH ≥ 9
Region C
START
SAMPLE
Basic compounds having low-pH instability
Initial separation
STEP 1
• ZORBAX SB-C18 or SB-C8
• pH 2 (1-3) 20-50 mM buffer
• T = 30°C (ambient to 80°C, SB-C18 to 90°C)
• Adjust % ACN for 0.5 < k < 20
STEP 1a
• Add 20 mM TEA or TEA acetate
• Re-adjust pH
Tailing Peaks
Poorly retained basic compounds
OR
Band spacing problems
STEP 2
Increase or decrease % of organic modifier by 5% (v/v)
STEP 2a
•Vary column temperature, up to 80°C; up to 90°C for ZORBAX SB-C18
Band spacing problems
STEP 1b
BASIC COMPOUNDS (Ion Pairing)
• Use MeOH organic modifier, 25-50 mM hexane sulfonic acid, 10 mM pH ≤ 3 buffer
• Adjust % MeOH for 0.5 < k < 20
STEP 3
• Change organic modifier (MeOH or THF)
• Adjust % organic for 0.5 < k < 20
• Restart at STEP [2]
STEP 1c
Vary temperature within recommended range for bonded phase
STEP 1d
Increase or decrease % of organic modifier by 5% (v/v)
STEP 4
• Change bonded-phase functionality to ZORBAX SB-CN, SB-C3 or SB-Phenyl
• Restart at STEP [1]
STEP 1e
• Change organic modifier (ACN or THF)
• Adjust for 0.5 < k < 20
STEP 5
• ZORBAX Eclipse XDB-C8 or XDB-C18
• pH 7 (6-9), 20-50 mM buffer
• T = 30°C (ambient to 40°C)
• Adjust % MeOH for 0.5 < k < 20
Poor retention or Band spacing problems
Poorly retained acidic compounds
STEP 6
Increase or decrease % of organic modifier by 5% (v/v)
STEP 6a
Vary temperature within recommended range for bonded phase
STEP 5b
ACIDIC COMPOUNDS (Ion Pairing)
• Use MeOH organic modifier, 25-50 mM tetrabutylammonium phosphate, 10 mM pH ≥ 7 buffer
• Adjust % MeOH for 0.5 < k < 20
STEP 7
• Change organic modifier (ACN or THF)
• Adjust for 0.5 < k < 20
• Restart at STEP [6]
STEP 5c
Vary temperature within recommended range for bonded phase
STEP 5d
Increase or decrease % of organic modifier by 5% (v/v)
STEP 8
• Change bonded-phase functionality to ZORBAX XDB-Phenyl or Bonus-RP
• Restart at STEP [5]
STEP 5e
• Change organic modifier (ACN or THF)
• Adjust for 0.5 < k < 20
STEP 9
• ZORBAX Extend-C18
• pH 10.5 (9-12); 5 mM ammonia, or TEA, or 10-50 mM organic buffer, or borate buffer
• T = 25°C (ambient to 40°C)
• Adjust % MeOH for 0.5 < k < 20
STEP 10
• Change organic modifier (ACN or THF)
• Adjust for 0.5 < k < 20
STEP 10a
Vary temperature within recommended range for bonded phase
Try different HPLC mode.
Call Agilent Technologies
Technical Support (800) 227-9770.

Adapted and updated by R.D. Ricker, B.A. Bidlingmeyer and J.J. Kirkland from J.J. Kirkland, LC/GC, 14 (1996) 486.

ZORBAX Columns for Analytical Chemistry

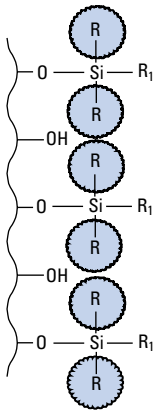
ZORBAX StableBond HPLC Columns

- Longest column lifetime and best reproducibility for low pH separations — down to pH 1!
- Patented stable column chemistry allows use at high temperature and low pH without degradation
- Six different bonded-phases provide broad selectivity — SB-C18, SB-C8, SB-CN, SB-Phenyl, SB-C3, SB-Aq
- High purity silica for superior peak shape
- Excellent first choice for method development



Agilent ZORBAX StableBond columns use patented, unique monofunctional silanes with bulky diisobutyl (SB-C18) or diisopropyl (SB-C8, SB-C3, SB-Phenyl, SB-CN and SB-Aq) side chain groups that sterically protect the key siloxane bond from hydrolytic attack at low pH. StableBond packing materials are not endcapped in order to provide exceptional stability and to maximize lifetime and reproducibility under acidic mobile phase conditions. The high purity, low acidity silica provides excellent peak shape with acidic, basic and neutral compounds so that StableBond columns are an excellent choice for low pH method development. ZORBAX StableBond columns are compatible with all common mobile phases, including very high aqueous mobile phases.

FIGURE 1
Sterically Protected StableBond Bonded Phase

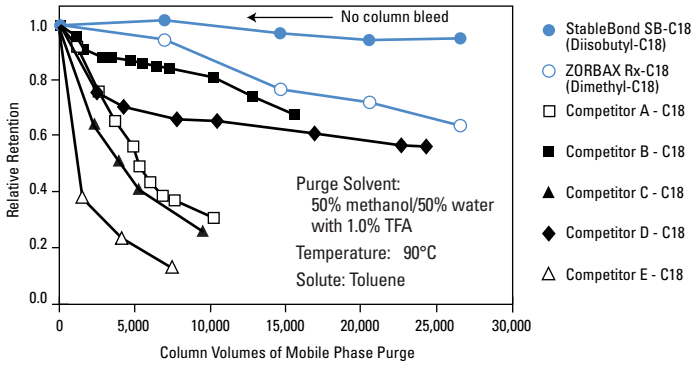


Bulky diisobutyl (C18) or diisopropyl (C8, C3, CN, Aq, Phenyl) side-chain groups are used to stabilize both long and short chain bonded phases.

R = Isopropyl, isobutyl (C18)

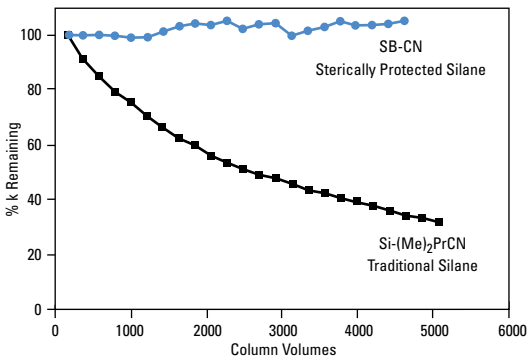
R_1 = C8, C18, CN, C3, etc.

FIGURE 2
StableBond SB-C18 Shows Excellent Stability at Low pH and High Temperature (pH 0.8, 90°C)



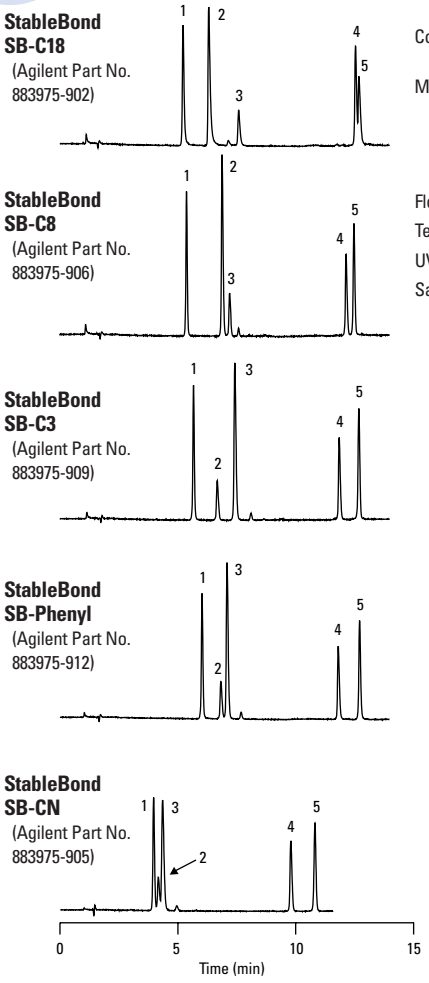
As an indicator of column breakdown, retention time of toluene was measured after purging the column with mobile phase. Only the StableBond SB-C18 is unchanged after 3 working months of use under these very low pH (0.8) and high temperature (90°C) conditions. ZORBAX Rx-C18 also provides a stable matrix, and can be used as an alternative selectivity to StableBond SB-C18.

FIGURE 3
Shorter Chain ZORBAX SB-CN is Also Stable at Low pH (pH 2.0, 50°C)



ZORBAX StableBond SB-CN, and the other short chain StableBond bonded phases, are also exceptionally stable at low pH. Conventional dimethyl CN and similar bonded phases lack this stability.

FIGURE 4
Five Different Bonded Phases Provide Selectivity Options



Columns: **ZORBAX StableBond**
4.6 x 150 mm, 5 µm

Mobile Phase: 0 - 100% B in 18.8 min
A: 50 mM NaH_2PO_4,
pH 2.5 in 95% H_2O / 5% ACN
B: 50 mM NaH_2PO_4,
pH 2.5 in 47% H_2O / 53% ACN

Flow Rate: 1.0 mL/min

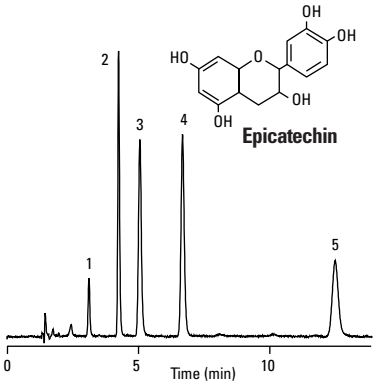
Temperature: 26°C

UV Detection: 254 nm

Sample:
1. Procaine
2. Lidocaine
3. d-Cinchonine
4. Butacaine
5. Tetracaine

SB-C3 is just one of the five different StableBond selectivity choices. In this example, optimum resolution is obtained with SB-C3. All are based on the same high purity Rx-SIL. Selectivity changes are therefore dependent only on the bonded phases making method development more reliable.

FIGURE 5
Components of Green Tea Separated on a Rapid Resolution StableBond SB-C8 Column



Column: **ZORBAX SB-C8**
4.6 x 150 mm, 3.5 µm
(Agilent Part No. 863953-906)

Mobile Phase: 75% 0.1% TFA : 25% MeOH

Flow Rate: 1.0 mL/min

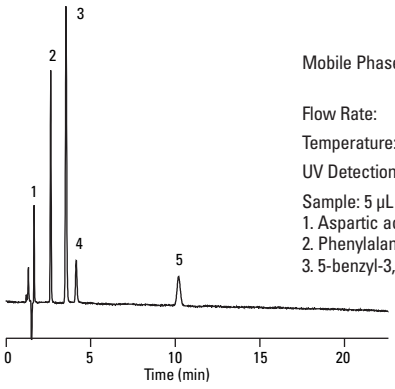
Temperature: 40°C

UV Detection: 280 nm

Sample:
1. Epigallocatechin
2. Epicatechin
3. Epigallocatechin gallate
4. Catechol
5. Epicatechin gallate

Nutraceuticals, such as the components of green tea, are quickly separated on a StableBond SB-C8 Rapid Resolution column.

FIGURE 6
Excellent Peak Shape



Column: **ZORBAX SB-C18**
4.6 x 150 mm, 5 µm
(Agilent Part No. 883975-902)

Mobile Phase: 85% 0.1% TFA
15% ACN

Flow Rate: 1.0 mL/min

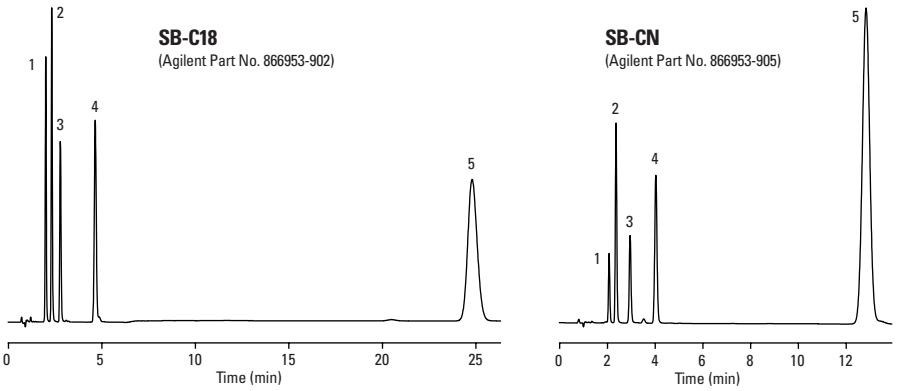
Temperature: 35°C

UV Detection: 210 nm

Sample: 5 µL
1. Aspartic acid
2. Phenylalanine
3. 5-benzyl-3, 6-dioxo-2-piperazineacetic acid
4. asp-phe
5. Aspartame

The high purity Rx-SIL used in the manufacturing of all StableBond columns provides excellent peak shape of basic and acidic compounds.

FIGURE 7
SB-CN Optimizes Retention and Resolution



Column: **ZORBAX SB** 4.6 x 75 mm, 3.5 µm
Mobile Phase: 30% ACN
70% NaH_2PO_4, pH 2.5
Flow Rate: 1.0 mL/min
Temperature: 35°C
Sample:
1. Estriol
2. Daidzen
3. Quercetin
4. Genistein
5. Diethylstilbestrol

The SB-CN column is used here to reduce analysis time by 50%. The retention of the most hydrophobic analyte is cut in half. At the same time retention of the more polar, early eluting peaks increases slightly.

ZORBAX StableBond Columns (80Å) Ordering Information

Column Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-CN USP L10	SB-C3	SB-Phenyl USP L11	SB-Aq	Price
Standard Columns and Bulk Packing									
Preparative	21.2 x 250	7	880975-102	880967-106	880975-105		880975-112		$2091
Semi-Preparative	9.4 x 250	5	880975-202	880967-201	880975-205	880975-209	880975-212		1099
Analytical	4.6 x 250	5	880975-902	880975-906	880975-905	880975-909	880975-912	880975-914	469
Analytical	4.6 x 150	5	883975-902	883975-906	883975-905	883975-909	883975-912	883975-914	414
Analytical	4.6 x 50	5	846975-902	846975-906				846975-914	304
Rapid Resolution (RR)	4.6 x 150	3.5	863953-902	863953-906	863953-905		863953-912		469
Rapid Resolution	4.6 x 75	3.5	866953-902	866953-906	866953-905		866953-912		414
Rapid Resolution	4.6 x 50	3.5	835975-902	835975-906	835975-905		835975-912		359
Solvent Saver	3.0 x 250	5	880975-302	880975-306	880975-305	880975-309	880975-312		469
Solvent Saver	3.0 x 150	5	883975-302	883975-306	883975-305	883975-309	883975-312		414
Solvent Saver Plus	3.0 x 150	3.5	863954-302	863954-306	863954-305		863954-312		469
Solvent Saver Plus	3.0 x 100	3.5		861954-306					435
Narrow Bore	2.1 x 150	5	883700-922	883700-906	883700-905	883700-909	883700-912		414
Narrow Bore	2.1 x 50	5	860975-902	860975-906	860975-905	860975-909	860975-912	860975-914	304
Narrow Bore RR*	2.1 x 150	3.5	830990-902	830990-906					469
Narrow Bore RR	2.1 x 50	3.5	871700-902	871700-906					359
MicroBore RR	1.0 x 150	3.5	863600-902	863600-906					505
MicroBore RR	1.0 x 50	3.5	865600-902	865600-906					388
MicroBore RR	1.0 x 30	3.5	861600-902	861600-906					301
Bulk Packing, 2 grams		5		820966-906	820966-905				136
Guard Cartridge, 2/pk	9.4 x 15	7	820675-115	820675-115	820675-124		820675-115		165
Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-920	820950-915	820950-916	820950-922	820950-917	820950-933	155
Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-926	821125-926	821125-924	821125-924	821125-926	821125-933	155
Guard Hardware Kit	9.4 x 15		840140-901	840140-901	840140-901	840140-901	840140-901		162
Guard Hardware Kit	4.6 x 12.5		820777-901	820777-901	820777-901	820777-901	820777-901	820777-901	106
Guard Hardware Kit	2.1 x 12.5		820777-901	820777-901	820777-901	820777-901	820777-901	820777-901	106

*RR: Rapid Resolution 3.5 µm columns.

Configurations not shown are available upon request. For more information on these and other columns consult the Agilent web site at **www.agilent.com**.
300Å StableBond columns are also available for protein and peptide separations. See page 26 for descriptions and ordering information

ZORBAX StableBond Columns (80Å) Ordering Information (cont'd)

Column Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	Price
Capillary Glass-lined Columns					
Capillary	0.5 x 250	5	5064-8258		$ 505
Capillary	0.5 x 150	5	5064-8256		455
Capillary RR*	0.5 x 150	3.5	5064-8262		505
Capillary RR	0.5 x 35	3.5	5064-8260		356
Capillary	0.3 x 250	5	5064-8257		505
Capillary	0.3 x 150	5	5064-8255		455
Capillary RR	0.3 x 150	3.5	5064-8261		505
Guard Cartridges	0.5 x 35	5	5064-8254		301
Guard Cartridges	0.3 x 35	5	5064-8253		301
Agilent Cartridge Columns					
Analytical	4.6 x 250	5	7995218-585	7995208-585	419
Analytical	4.6 x 150	5	7995218-595	7995208-595	386
Rapid Resolution	4.6 x 75	3.5	7995218-344	7995208-344	386
Guard Cartridge, 10/pk	4.0 x 4.0	5	7995118-504	7995118-504	228
Cartridge Holder			5021-1845	5021-1845	192
High Throughput Cartridge Columns (requires Hardware Kit 820222-901)					
Rapid Resolution Cartridge	4.6 x 30	3.5	833975-902	833975-906	194
Rapid Resolution Cartridge, 3/pk	4.6 x 30	3.5	833975-932	833975-936	547
Rapid Resolution Cartridge	4.6 x 15	3.5	831975-902	831975-906	155
Rapid Resolution Cartridge, 3/pk	4.6 x 15	3.5	831975-932	831975-936	436
Rapid Resolution Cartridge	2.1 x 30	3.5	873700-902	873700-906	194
Rapid Resolution Cartridge, 3/pk	2.1 x 30	3.5	873700-932	873700-936	547
Rapid Resolution Cartridge	2.1 x 15	3.5	875700-902	875700-906	155
Rapid Resolution Cartridge, 3/pk	2.1 x 15	3.5	875700-932	875700-936	436
High Throughput Cartridge Column Hardware Kit			820222-901	820222-901	162
CombiHT High Throughput Preparative Columns (endfittings required)					
CombiHT	21.2 x 150	5	870150-902	870150-906	1665
CombiHT	21.2 x 100	5	870100-902	870100-906	1248
CombiHT	21.2 x 50	5	870050-902	870050-906	827
CombiHT End Fittings (required for use)			820400-901	820400-901	203

*RR: Rapid Resolution 3.5 µm columns.

Configurations not shown are available upon request.
For more information on these and other columns consult the Agilent web site at **www.agilent.com**.

300Å StableBond columns are also available for protein and peptide separations. See page 26 for descriptions and ordering information

ZORBAX StableBond Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits*	pH Range*	Endcapped	Carbon Load
ZORBAX SB-C18	80Å	180 m^2/g	90°C	1.0 - 8.0	No	10%
ZORBAX SB-C8	80Å	180 m^2/g	80°C	1.0 - 8.0	No	5.5%
ZORBAX SB-C3	80Å	180 m^2/g	80°C	1.0 - 8.0	No	4.0%
ZORBAX SB-CN	80Å	180 m^2/g	80°C	1.0 - 8.0	No	4.0%
ZORBAX SB-Phenyl	80Å	180 m^2/g	80°C	1.0 - 8.0	No	5.5%
ZORBAX SB-Aq	80Å	180 m^2/g	80°C	1.0 - 8.0	No	proprietary

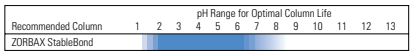
*StableBond columns are designed for optimal use at low pH. At pH 6-8, highest column stability for all silica-based columns is obtained by operating at temperatures <40°C and using low buffer concentrations in the range of 0.01-0.02M. At mid-range pH, Eclipse XDB and Bonus-RP are recommended.

To order or for technical assistance, call Agilent Technologies at 800-227-9770, or contact your local authorized Agilent distributor.

ZORBAX Columns for Analytical Chemistry

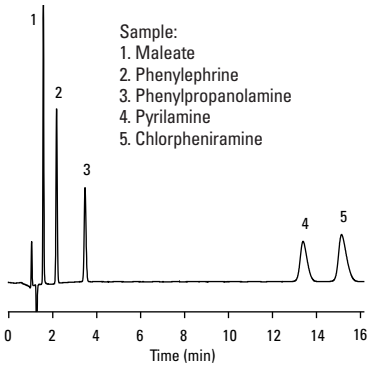
ZORBAX SB-Aq HPLC Columns

- Recommended for high resolution analysis of very polar compounds
- Achieve high retention in highly aqueous mobile phases
- Obtain reproducible retention without phase collapse
- Provide exceptional column Lifetime at low pH



ZORBAX SB-Aq columns are an ideal choice to increase the retention of many difficult acidic, basic and polar compounds. More retention may be obtained than on many conventional ODS HPLC columns. Polar compounds can be analyzed with excellent reproducibility and peak shape because the SB-Aq columns have a hydrophilic surface that inhibits phase collapse, even with 100% aqueous mobile phases. ZORBAX StableBond technology adds exceptional durability and stability at low pH and high temperature — down to pH 1 and up to 80°C with the SB-Aq column.

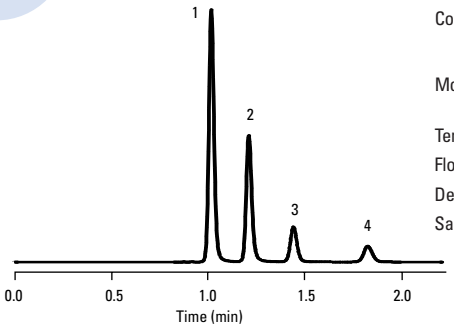
FIGURE 1
Excellent Retention in a High Aqueous Mobile Phase on the SB-Aq Column



Column:	**ZORBAX SB-Aq** 4.6 x 150 mm, 5 µm (Agilent Part No. 883975-914)
Mobile Phase:	90% 0.2% TFA 10% ACN
Temperature:	25°C
Flow Rate:	1.5 mL/min
Detection:	UV 254 nm

The basic compounds analyzed here are very polar and difficult to retain and separate without using a gradient method. The SB-Aq column increases the retention of the early eluting compounds and resolves all components using a simple isocratic, highly aqueous mobile phase.

FIGURE 2
Separation of Water Soluble Vitamins on the SB-Aq Column without Ion Pairing



Column:	**ZORBAX SB-Aq** 4.6 x 150 mm, 5 µm (Agilent Part No. 883975-914)
Mobile Phase:	95% 0.1% TFA 5% MeOH
Temperature:	35°C
Flow Rate:	2.0 mL/min
Detection:	UV 254 nm
Sample:	1. Thiamine (B1) 2. Nicotinic Acid 3. Pyridoxine (B6) 4. Niacinamide

The SB-Aq column is used with a simple mobile phase (no ion-pair reagents) to achieve sufficient retention and high resolution of these B vitamins.

ZORBAX SB-Aq Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped
ZORBAX SB-Aq	80Å	180 m^2/g	80°C	1.0 - 8.0	No

ZORBAX StableBond Aq (SB-Aq) Column Ordering Information

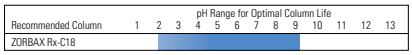
Column Description	Dimension (mm)	Particle Size (µm)	Part Number	Price
Analytical	4.6 x 250	5	880975-914	$ 469
Analytical	4.6 x 150	5	883975-914	414
Analytical	4.6 x 50	5	846975-914	304
Narrow Bore	2.1 x 50	5	860975-914	304
Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-933	155
Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-933	155
Guard Hardware Kit			820777-901	106

Configurations not shown are available upon request.
For more information on these and other columns consult the Agilent web site at **www.agilent.com**.

ZORBAX Columns for Analytical Chemistry

ZORBAX Rx HPLC Columns

- Rx-C18 is recommended for alternate selectivity at low pH relative to StableBond SB-C18; for higher temperature applications, StableBond is recommended. This column has a higher carbon load than SB-C18 columns (12% vs. 10%).
- Rx-C18 offers high stability and good peak shape for low pH applications
- Rx-C18 is manufactured using dimethyloctadecylsilane, is non-endcapped and provides excellent stability up to pH 9



- Rx-C8 is the same product as SB-C8

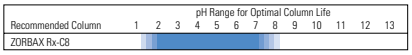
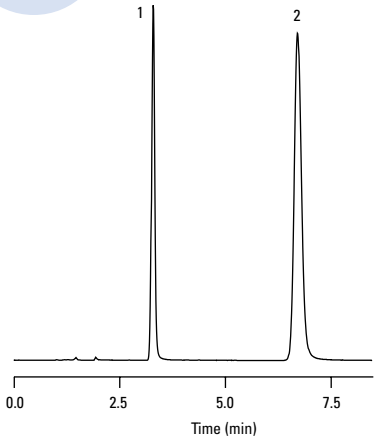


FIGURE 1
Analysis of Diazepam on Rx-C18



Column: **ZORBAX Rx-C18**
3.0 x 250 mm, 5 µm
(Agilent Part No. 880967-302)

Mobile Phase: 35% H_2O
65% MeOH

Flow Rate: 0.5 mL/min

Sample: 1. Ethylparaben
2. Diazepam

An Rx-C18 column is used for this USP analysis of diazepam and the internal standard ethylparaben. The Solvent Saver 3.0 mm i.d. Rx-C18 column reduces solvent usage by 60% over what would be used if the analysis were done on a 4.6 x 250 mm column.

ZORBAX Rx Column Ordering Information

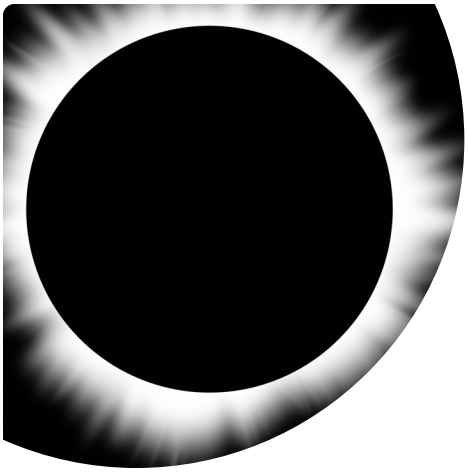
Column Description	Size (mm)	Particle Size (µm)	Rx-C18	Rx-C8	Price
Standard Columns					
Preparative	21.2 x 250	7	880967-102	880967-106	$ 2091
Semi-Preparative	9.4 x 250	5	880967-202	880967-201	1099
Analytical	4.6 x 250	5	880967-902	880967-901	469
Analytical	4.6 x 150	5	883967-902	883967-901	414
Rapid Resolution	4.6 x 150	3.5	863967-902	863953-906	469
Rapid Resolution	4.6 x 75	3.5	866967-902	866953-906	414
Solvent Saver	3.0 x 250	5	880967-302	880975-306	469
Solvent Saver	3.0 x 150	5	883967-302	883975-306	414
Narrow Bore	2.1 x 150	5	883700-902	883700-906	414
Bulk Packing, 2 grams		5		820966-906	136
Guard Cartridge packed with Rx/SB-C8, 2/pk	9.4 x 15	7	820675-115	820675-115	165
Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-914	820950-913	155
Guard Cartridge packed with Eclipse XDB-C8, 4/pk	2.1 x 12.5	5	821125-926	821125-926	155
Guard Hardware Kit	9.4 x 15		840140-901	840140-901	162
Guard Hardware Kit	4.6 x 12.5		820777-901	820777-901	106
Guard Hardware Kit	2.1 x 12.5		820777-901	820777-901	106

NOTE: Rx-C8 is the same product as StableBond SB-C8
Configurations not shown are available upon request.
For more information on these and other columns consult the Agilent web site at **www.agilent.com**.

To order or for technical assistance, call Agilent Technologies at 800-227-9770, or contact your local authorized Agilent distributor.

ZORBAX Rx Column Specifications

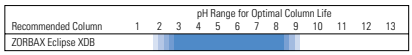
Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Rx-C18	80Å	180 m^2/g	60°C	2.0 - 9.0	No	12%
ZORBAX Rx-C8	80Å	180 m^2/g	80°C	1.0 - 8.0	No	5.5%



ZORBAX Columns for Analytical Chemistry

ZORBAX Eclipse XDB HPLC Columns

- Excellent peak shape for bases, acids and neutrals
- Long lifetime at mid pH with extra dense bonding and double endcapping
- Extended pH range — pH 2 - 9



The Agilent ZORBAX Eclipse XDB columns — C18, C8 and Phenyl — are designed to provide good peak shape for basic compounds at mid pH. In the mid-pH region residual silanols are more active and tailing interactions are more likely. To overcome these interactions Eclipse XDB columns are eXtra Densely Bonded and then double endcapped through a proprietary process to cover as many active silanols as possible. Column lifetime at mid pH is enhanced through the dense bonding and the choice of the thick-walled Rx-SIL support, which resists silica dissolution in the mid-pH range. As a result, the Eclipse XDB columns can be used over the pH range 2 - 9, with an optimum lifetime from pH 3 - 8. The Eclipse XDB columns also provide an alternate selectivity to the StableBond, Bonus-RP and Extend-C18 columns.

FIGURE 1
Extra Densely Bonded and Double Endcapped Eclipse XDB Bonded Phase

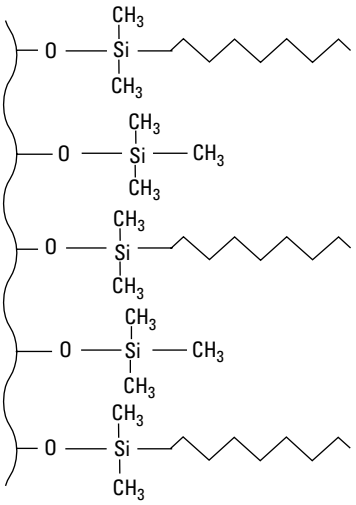
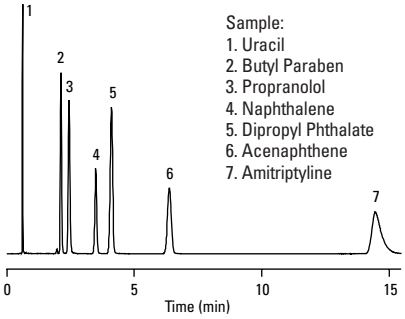


FIGURE 3
Good Peak Shape for Acids, Bases, and Neutrals on ZORBAX Eclipse XDB at Mid pH

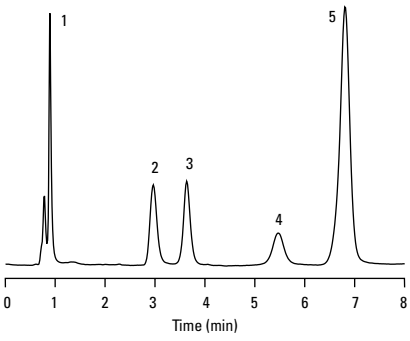


Column: **ZORBAX Rapid Resolution Eclipse XDB-C8**
4.6 x 75 mm, 3.5 µm
(Agilent Part No. 966967-906)
Mobile Phase: 35% 20 mM NaH_2PO_4, pH 7.0
65% MeOH
Flow Rate: 1.0 mL/min
Temperature: 35°C
Detection: UV 254 nm

In the mid-pH region, residual silanols can interact more strongly with analytes, resulting in asymmetric peaks. The Eclipse XDB column provides good peak shape in this difficult pH range.

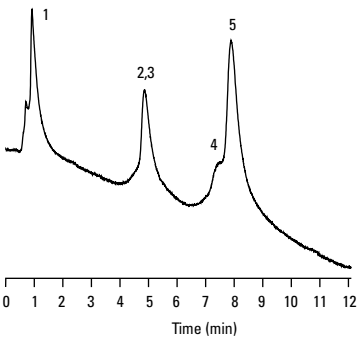
FIGURE 2
Column Stability Testing

Eclipse XDB-C8
Sol-type
After 1843 Column Volumes



Column: 4.6 x 150 mm, 5µm
Mobile Phase: 60% ACN
40% 25 mM phosphate buffer, pH 7.0
Flow Rate: 1.5 mL/min
Temperature: 60°C

Waters Symmetry C8
SIL-type
After 1826 Column Volumes



Sample: Tricyclic Antidepressants
1. Uracil
2. Nortriptyline
3. Doxepin
4. Amitriptyline
5. Trimipramine

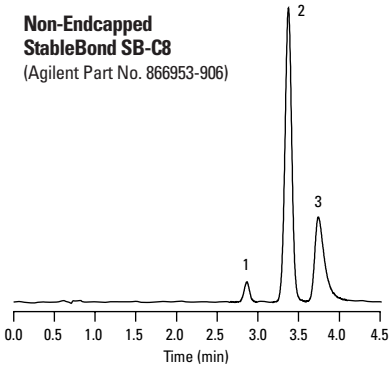
Double endcapping, dense bonding, and the durable Rx-SIL particles (sol-type) combine to provide long lifetime at pH 7.0 when compared to single endcapped sil-gel columns, such as the Waters Symmetry C8 column used here. The conditions used for this test — high temperature (60°C) and high salt concentration (250 mM), accelerate the dissolution of silica, causing premature failure of the sil-gel type column.

ZORBAX Eclipse XDB Column Specifications

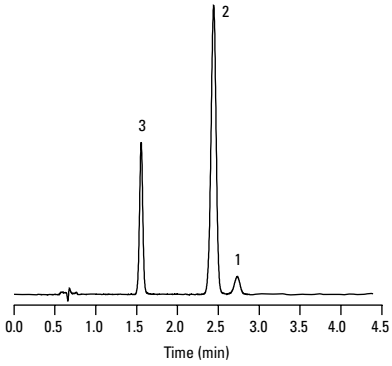
Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Eclipse XDB-C18	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	10%
ZORBAX Eclipse XDB-C8	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	7.6%
ZORBAX Eclipse XDB-Phenyl	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	7.2%

FIGURE 4
Selectivity Changes for Basic Compounds with Eclipse XDB and StableBond

Non-Endcapped StableBond SB-C8
(Agilent Part No. 866953-906)



Double Endcapped Eclipse XDB-C8
(Agilent Part No. 966967-906)



Columns: **Rapid Resolution** 4.6 x 75 mm, 3.5 µm
Mobile Phase: 70% 25 mM NaH_2PO_4, pH 3.0
30% Methanol
Flow Rate: 1.0 mL/min
Temperature: 35°C
Sample: 1. Barbital
2. Sulfamethoxazole
3. Caffeine

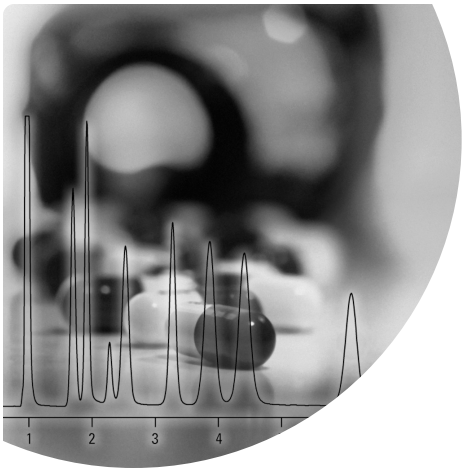
Eclipse XDB and StableBond columns are based on the same silica but have different bonding and endcapping. Therefore, they can have very different selectivity for the same sample under the same conditions, as this example shows.

ZORBAX Eclipse XDB Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7	XDB-Phenyl USP L11	Price
Standard Columns and Bulk Packings						
Semi-Prep	9.4 x 250	5	990967-202	990967-206		$ 1099
Analytical	4.6 x 250	5	990967-902	990967-906	990967-912	469
Analytical	4.6 x 150	5	993967-902	993967-906	993967-912	414
Analytical	4.6 x 50	5	946975-902	946975-906		304
Rapid Resolution	4.6 x 150	3.5	963967-902	963967-906	963967-912	469
Rapid Resolution	4.6 x 75	3.5	966967-902	966967-906	966967-912	414
Rapid Resolution	4.6 x 50	3.5	935967-902	935967-906	935967-912	359
Solvent Saver	3.0 x 250	5	990967-302	990967-306	990967-312	469
Solvent Saver	3.0 x 150	5	993967-302	993967-306	993967-312	414
Solvent Saver Plus	3.0 x 150	3.5	963954-302	963954-306	963954-312	469
Narrow Bore	2.1 x 150	5	993700-902	993700-906	993700-912	414
Narrow Bore	2.1 x 50	5	960967-902	960967-906	960967-912	304
Narrow Bore RR*	2.1 x 150	3.5	930990-902	930990-906		469
Narrow Bore RR	2.1 x 50	3.5	971700-902	971700-906		359
MicroBore RR	1.0 x 150	3.5	963600-902	963600-906		505
MicroBore RR	1.0 x 50	3.5	965600-902	965600-906		388
MicroBore RR	1.0 x 30	3.5	961600-902	961600-906		301
Bulk Packing, 2 grams		5	920966-902			136
Guard Cartridges, 4/pk	4.6 x12.5	5	820950-925	820950-926	820950-927	155
Guard Cartridges, 4/pk	2.1 x12.5	5	821125-926	821125-926	821125-926	155
Guard Hardware Kit			820777-901	820777-901	820777-901	106
Agilent Cartridge Columns						
Analytical	4.6 x 250	5	7995118-585	7995108-585		419
Analytical	4.6 x 150	5	7995118-595	7995108-595		386
Rapid Resolution	4.6 x 75	3.5	7995118-344	7995108-344		386
Solvent Saver	3.0 x 75	3.5	7995230-344			340
Guard Cartridges, 10/pk	4.0 x 4	5	7995118-504	7995118-504		228
Cartridge Holder			5021-1845	5021-1845		192
High Throughput Cartridge Columns (requires Hardware Kit 820222-901)						
Rapid Resolution Cartridge	4.6 x 30	3.5	933975-902	933975-906		194
Rapid Resolution Cartridge, 3/pk	4.6 x 30	3.5	933975-932	933975-936		547
Rapid Resolution Cartridge	4.6 x 15	3.5	931975-902	931975-906		155
Rapid Resolution Cartridge, 3/pk	4.6 x 15	3.5	931975-932	931975-936		436
Rapid Resolution Cartridge	2.1 x 30	3.5	973700-902	973700-906		194
Rapid Resolution Cartridge, 3/pk	2.1 x 30	3.5	973700-932	973700-936		547
Rapid Resolution Cartridge	2.1 x 15	3.5	975700-902	975700-906		155
Rapid Resolution Cartridge, 3/pk	2.1 x 15	3.5	975700-932	975700-936		436
Hardware Kit for High Throughput Columns			820222-901	820222-901		162
CombiHT Columns (end fittings required)						
CombiHT	21.2 x 150	5	970150-902	970150-906		1665
CombiHT	21.2 x 100	5	970100-902	970100-906		1248
CombiHT	21.2 x 50	5	970050-902	970050-906		827
CombiHT End Fittings (2) (required for use)			820400-901	820400-901		203
Capillary Glass-lined Columns						
Capillary	0.5 x 250	5	5064-8286			505
Capillary	0.5 x 150	5	5064-8287			455
Capillary RR	0.5 x 150	3.5	5064-8288			505
Capillary RR	0.5 x 35	3.5	5064-8298			356
Capillary	0.3 x 250	5	5064-8269			505
Capillary	0.3 x 150	5	5064-8291			455
Capillary RR	0.3 x 150	3.5	5064-8271			505
Guard Cartridges	0.5 x 35	5	5064-8296			301
Guard Cartridges	0.3 x 35	5	5064-8297			301

*RR: Rapid Resolution 3.5 µm columns.

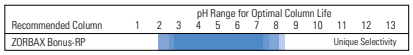
Configurations not shown are available upon request.
For more information on these and other columns consult the Agilent web site at **www.agilent.com**.



ZORBAX Columns for Analytical Chemistry

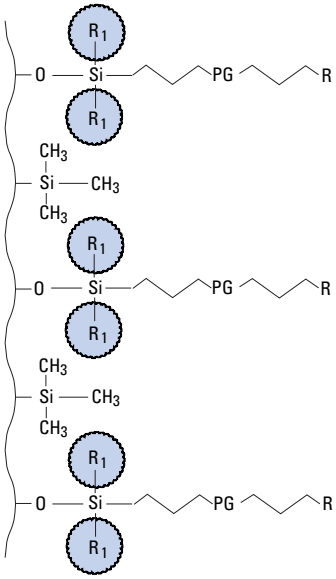
ZORBAX Bonus-RP HPLC Columns

- Excellent peak shape for challenging basic compounds at low and mid pH
- Unique reversed-phase selectivity
- Novel bonding technology with embedded polar group and steric protection
- Usable in 100% aqueous mobile phases



The Agilent ZORBAX Bonus-RP column has a polar amide group embedded in a long alkyl chain. This novel bonding reduces interactions between basic compounds and the silica support, improving peak shape for the most difficult basic compounds. Peak shape and column lifetime are further improved by triple endcapping. In addition, diisopropyl side groups provide steric protection against acid hydrolysis for good lifetime at low pH. The Bonus-RP column provides an alternate selectivity to C18 and C8 alkyl bonded phases.

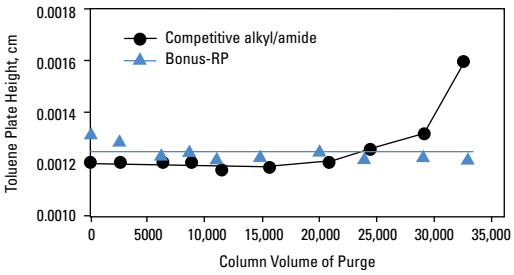
FIGURE 1
Unique, Polar Alkyl Bonus-RP Bonded Phase



Triple Endcapped
PG = polar amide group
R_1 = sterically protecting isopropyl group
R = C14 alkyl chain

FIGURE 2
ZORBAX Bonus-RP is Stable at Mid and Low pH

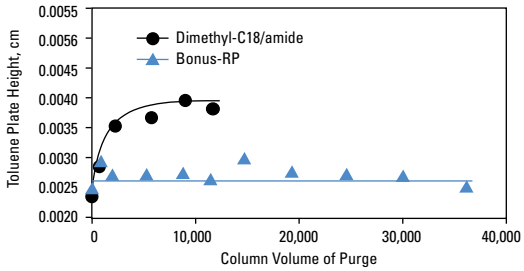
Mid-pH Stability



Columns: 4.6 x 150 mm
Mobile Phase: 60% 25 mM phosphate buffer, pH 7.0
40% ACN
Flow Rate: 1.5 mL/min
Temperature: 23°C

Triple endcapping of Bonus-RP enhances stability at pH 7. Each 10,000 column volumes is equivalent to approximately one working month.

Low pH Stability



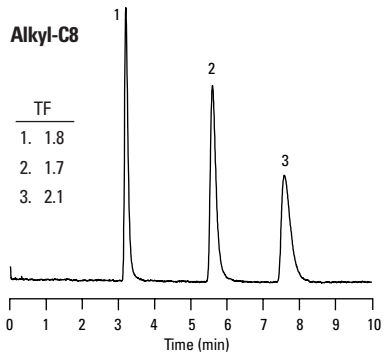
Columns: 4.6 x 150 mm
Mobile Phase Aging: 50% MeOH
50% 0.1% TFA
Flow Rate: 1.0 mL/min
Temperature: 60°C
Mobile Phase Test: 80% MeOH
20% H_2O
Flow Rate: 1.0 mL/min
Temperature: 23°C

Sterically protecting side groups provide good low pH stability and longer column lifetime than similar polar alkyl bonded phases.

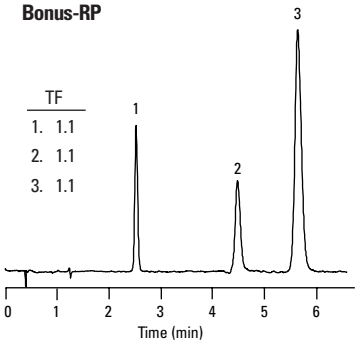
ZORBAX Bonus-RP Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Bonus-RP	80Å	180 m^2/g	60°C	2.0 - 9.0	Triple	9.5%

FIGURE 3
Improved Peak Shape of Basic Compounds Using Bonus-RP



Column:	4.6 x 150 mm, 5 µm
Mobile Phase:	75% 25 mM NH_4OAc, pH 5.5 25% ACN
Flow Rate:	1.5 mL/min
Temperature:	40°C
Detection:	254 nm
Sample:	1. Doxylamine 2. Chlorpheniramine 3. Triprolidine

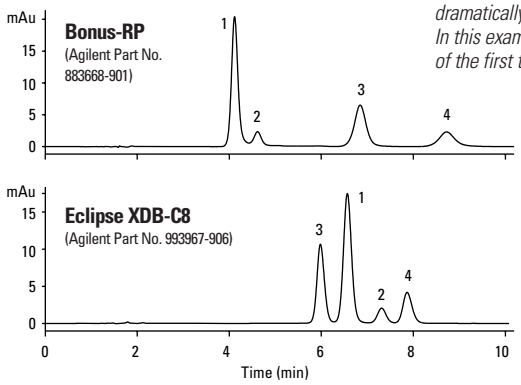


Column:	4.6 x 150 mm, 5 µm (Agilent Part No. 883668-901)
Mobile Phase:	80% 25 mM NH_4OAc, pH 5.5 20% ACN
Flow Rate:	1.5 mL/min
Temperature:	40°C
Detection:	254 nm
Sample:	1. Doxylamine 2. Chlorpheniramine 3. Triprolidine

Bonus-RP eliminates peak tailing of these basic compounds in comparison to a typical alkyl C8 bonded phase. In the mid-pH region, residual silanols can interact more strongly with basic compounds to cause peak tailing. The polar group in the Bonus-RP bonded phase eliminates peak tailing of these basic compounds by reducing interactions with residual silanols.

FIGURE 4
ZORBAX Bonus-RP Provides Unique Selectivity

Peak elution order can change dramatically when using Bonus-RP. In this example, the elution order of the first three peaks change.



Column:	4.6 x 150 mm	Injection vol:	3 µL
Mobile Phase:	75% 25 mM Na Citrate, pH 6 25% MeOH	Sample:	Cephalosporins 1. cephalexin 2. cephaclor 3. cephuroxime 4. cephoxitin
Flow Rate:	1.0 mL/min		
Temperature:	Ambient		
Detection:	254 nm		

ZORBAX Bonus-RP Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	Part Number	Price
Analytical	4.6 x 250	5	880668-901	$ 469
Analytical	4.6 x 150	5	883668-901	414
Rapid Resolution	4.6 x 150	3.5	863668-901	469
Rapid Resolution	4.6 x 75	3.5	866668-901	414
Narrow Bore	2.1 x 150	5	883725-901	414
Narrow Bore	2.1 x 50	5	861971-901	304
Rapid Resolution	2.1 x 50	3.5	861700-901	359
MicroBore RR*	1.0 x 150	3.5	863608-901	505
MicroBore RR	1.0 x 50	3.5	865608-901	388
MicroBore RR	1.0 x 30	3.5	861608-901	301
Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-928	155
Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-928	155
Guard Hardware Kit			820777-901	106

*RR: Rapid Resolution 3.5 µm columns.

Configurations not shown are available upon request. For more information on these and other columns consult the Agilent web site at **www.agilent.com**.

To order or for technical assistance, call Agilent Technologies at 800-227-9770, or contact your local authorized Agilent distributor.

ZORBAX Columns for Analytical Chemistry

ZORBAX Extend-C18 HPLC Columns

- High efficiency and long life at high pH — up to pH 11.5
- Unique bidentate bonding and double endcapping provides high pH stability
- More efficiency and better peak shape than polymer-based columns
- Improve retention, resolution and peak shape of basic compounds
- High sensitivity for LC/MS separations of peptides

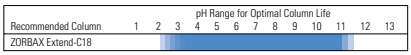
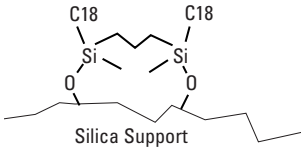


FIGURE 1
New Bidentate C18-C18 Bonding for Extend-C18 Bonded Phase

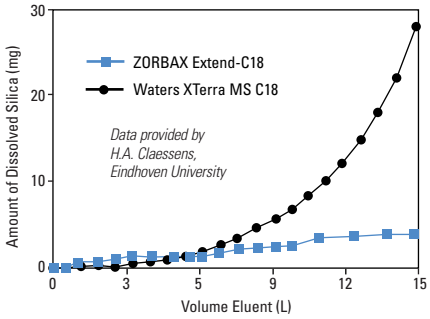


The combination of the unique bidentate structure and double endcapping permits long life at high pH.

The Agilent ZORBAX Extend-C18 column uses a new bidentate C18-C18 column bonding technology to make high pH separations a good choice with a silica-based column. At high pH, non-charged basic compounds will not interact with the silica, so it is possible to do separations with high efficiency, superior peak shape and improved resolution. High pH separations are also the best choice for compounds that are more stable or more soluble in high pH solutions. Some of the mobile phase buffer options for high pH include triethylamine, pyrrolidine, glycine, borate and ammonium hydroxide. Ammonium hydroxide at pH 10.5 is an excellent mobile phase modifier for the LC/MS of peptides and small molecules with improved sensitivity compared with TFA containing mobile phase at low pH. The Extend-C18 column is stable from pH 2 - 11.5 with good peak shape for all types of compounds. Extend-C18 columns also provide an additional selectivity choice at low pH.[1]

[1] Kirkland, J.J., J.B. Adams, Jr., M.A. van Straten and H.A. Claessens, (1998) Analytical Chemistry, 70, 4344-4352.

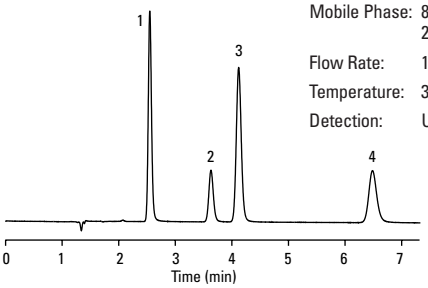
FIGURE 2
Long Life at High pH with Extend-C18



Column: **ZORBAX Extend-C18**
4.6 x 150 mm
Mobile Phase: 20% Methanol
80% 0.1 M carbonate buffer, pH 10.0
Flow Rate: 1.0 mL/min
Temperature: Ambient

At high pH, columns will fail due to silica dissolution. The example here shows extended lifetime of ZORBAX Extend-C18 at high pH in comparison to Waters XTerra MS C18. This was measured by the amount of dissolved silica.

FIGURE 3
Extend-C18 Provides Good Peak Shape at Low pH



Column: **ZORBAX Extend-C18**
4.6 x 150 mm, 5µm
(Agilent Part No. 773450-902)
Mobile Phase: 80% 25 mM NaH_2PO, pH 3.0
20% Methanol
Flow Rate: 1.0 mL/min
Temperature: 35°C
Detection: UV 254 nm

Sample:
1. Theobromine
2. Dimethylxanthine
3. Theophylline
4. Caffeine

These basic compounds are separated on the Extend-C18 at low pH with excellent peak shape. The Extend-C18 column can be used at high and low pH.

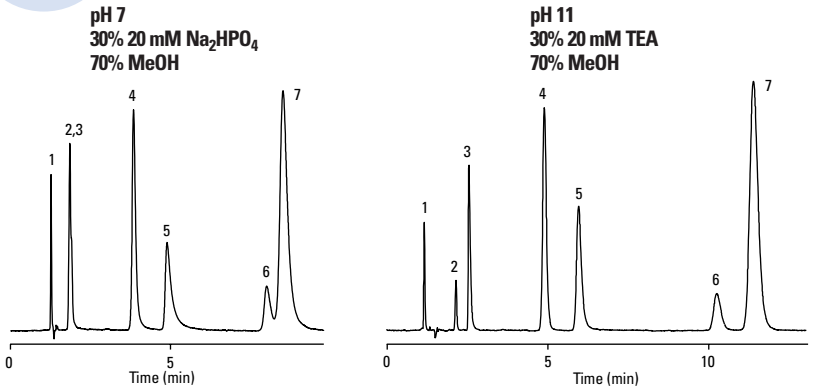
See the 300Extend-C18 section on page 28 for additional examples.

ZORBAX Extend-C18 Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Extend-C18	80Å	180 m^2/g	60°C*	2.0 - 11.5	Double	12.5%

**Temperature limits are 60°C up to pH 8, 40°C for pH 8 - 11.5.*

FIGURE 4
Improved Retention, Resolution and Peak Shape of Basic Antihistamines on Extend-C18 at High pH



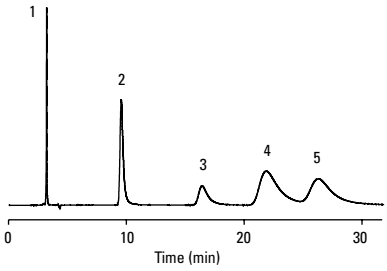
Pseudoephedrine and scopolamine are difficult to retain at low and mid pH. Pseudoephedrine is often analyzed by ion exchange methods. The Extend-C18 column retains these compounds in a noncharged form at high pH and improves resolution.

Column: **ZORBAX Extend-C18**
4.6 x 150 mm, 5 µm
(Agilent Part No. 773450-902)
Mobile Phase: See figure
Flow Rate: 1.0 mL/min
Temperature: R.T.
Detection: 254 nm

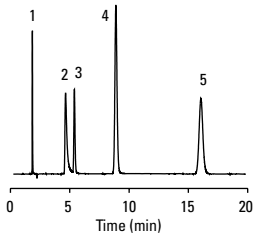
Sample:		pKa
	1. Maleate	
	2. Scopolamine	7.6
	3. Pseudoephedrine	9.8
	4. Doxylamine	9.2
	5. Chlorpheniramine	9.1
	6. Triprolidine	6.5
	7. Diphenhydramine	9.0

FIGURE 5
High Efficiency Separation of Basic Compounds at High pH on Extend-C18

Polymeric-Based Column, 4.0 x 250 mm, 5 µm
Flow Rate: 0.5 mL/min
Pressure: 142 bar



Extend-C18, 4.6 x 250 mm, 5 µm
(Agilent Part No. 770450-902)
Flow Rate: 1.0 mL/min
Pressure: 213 bar



Mobile Phase: 65% 20 mM TEA, pH 11
35% MeOH
Temperature: RT
Detection: UV 254 nm
Sample:
1. Pyridoxine
2. Pyridine
3. N-methylbenzylamine
4. Procainamide
5. N-acetylprocainamide

The Extend-C18, a silica based column, provides greater efficiency, improved peak shape and faster analysis at high pH in comparison to a polymeric bonded phase. A lower flow rate had to be used for the polymeric column due to a pressure limitation of 180 bar.

To order or for technical assistance, call Agilent Technologies at 800-227-9770, or contact your local authorized Agilent distributor.

ZORBAX Extend-C18 Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	Part Number	Price
Analytical	4.6 x 250	5	770450-902	$ 551
Analytical	4.6 x 150	5	773450-902	490
Analytical	4.6 x 50	5	746450-902	357
Rapid Resolution	4.6 x 150	3.5	763953-902	551
Rapid Resolution	4.6 x 75	3.5	766953-902	490
Rapid Resolution	4.6 x 50	3.5	735953-902	423
Narrow Bore	2.1 x 150	5	773700-902	490
Narrow Bore	2.1 x 50	5	760450-902	357
Narrow Bore RR*	2.1 x 50	3.5	735700-902	423
MicroBore RR	1.0 x 150	3.5	763600-902	505
MicroBore RR	1.0 x 50	3.5	765600-902	388
MicroBore RR	1.0 x 30	3.5	761600-902	301
Bulk Packing, 2 grams		5	820966-402	139
Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-930	184
Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-930	184
Guard Hardware Kit			820777-901	106

*RR: Rapid Resolution 3.5 µm columns.

Configurations not shown are available upon request.
300Å Extend-C18 columns are also available for peptide separations.
See page 28 for descriptions and ordering information

Additional ZORBAX Analytical HPLC Columns

Listed here, for your convenience, are additional ZORBAX Analytical column part numbers. For further information, consult the Agilent 2000/2001 Catalog or visit our web site at **www.agilent.com/chem**.

- ZORBAX Method Validation Kits
- ZORBAX Method Development Kits
- Original ZORBAX Columns
- ZORBAX Normal Phase Columns

ZORBAX Method Validation Kits

ZORBAX Validation Kits are supplied to customers who need the same HPLC column type (bonded phase, particle size, configuration) but from different manufacturing lots. To request columns from different lots, contact Agilent Technologies or your local distributor using the following procedure.

1. Request Validation Kits (columns from different lots) by using Part Number 899999-888.
2. Indicate the Part Number of the current column you are using.
3. Indicate the Lot Number of the current column you are using.
4. Indicate the number of additional columns needed from different lots (e.g.: you have a current column and may need two other columns from additional lots).
5. Your customer service representative will issue a Special Products Quote (SPQ) number, price and anticipated delivery date for your order. This process may take 1 - 2 working days, and delivery is usually within 1 - 3 weeks from time of order, depending on availability.

ZORBAX Method Development Kits

Description	Agilent Part No.	Price
Traditional StableBond Kit: Includes 3 columns, 4.6 x 150 mm packed with SB-C18, 5 µm, SB-CN, 5 µm and SB-Phenyl, 5 µm	5183-4624	$ 1056
Fast StableBond Kit: Includes 3 columns, 4.6 x 75 mm packed with SB-C18, 3.5 µm, SB-CN, 3.5 µm and SB-Phenyl, 3.5 µm	5183-4625	1056
Traditional Eclipse XDB Kit: Includes 3 columns, 4.6 x 150 mm packed with XDB-C18, 5 µm, XDB-C8, 5 µm and XDB-Phenyl, 5 µm	5183-4626	1056
Fast Eclipse XDB Kit: Includes 3 columns, 4.6 x 75 mm packed with XDB-C18, 3.5 µm, XDB-C8, 5 µm and XDB-Phenyl, 3.5 µm	5183-4627	1056

Original ZORBAX Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	ODS (C18) USP L1	C8 USP L7	Phenyl USP L11	CN USP L10	TMS USP L13	Price
Standard Columns								
Preparative	21.2 x 250	7	880952-102	880952-106		880952-105		$ 2091
Semi-Preparative	9.4 x 250	5	880952-202	880952-206	880952-212	880952-205		1099
Analytical (Endcapped)	4.6 x 250	5	880952-702	880952-706	880952-712	884950-507	880952-710	469
Analytical (Non-endcapped)	4.6 x 250	5	884950-543					469
Analytical	4.6 x 150	5	883952-702	883952-706	883952-712	884950-526	883952-710	414
Solvent Saver	3.0 x 250	5	880952-302					469
Solvent Saver	3.0 x 150	5	883952-302					414
Guard Cartridges, 2/pk	9.4 x 15	7	820675-115	820675-115	820675-115	820675-124		165
Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-902	820950-906	820950-912	820950-905	820950-905	155
Guard Hardware Kit	9.4 x 15		840140-901	840140-901	840140-901	840140-901	840140-901	162
Guard Hardware Kit	4.6 x 12.5		820777-901	820777-901	820777-901	820777-901	820777-901	106

Configurations not shown are available upon request.

ZORBAX Normal Phase Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	Rx-SIL USP L3	SIL USP L3	CN USP L10	NH_2	Carbohydrate Analysis	Price
Preparative	21.2 x 250	7	880975-101	880952-101	880952-105	880952-108		$ 2091
Semi-Preparative	9.4 x 250	5	880975-201	880952-201	880952-205	880952-208		1099
Analytical	4.6 x 250	5	880975-901		880952-705	880952-708	840300-908	469
Analytical	4.6 x 250	5		880952-701				430
Analytical	4.6 x 150	5	883975-901		883952-705	883952-708	843300-908	414
Analytical	4.6 x 150	5		883952-701				359
Narrow Bore	2.1 x 150	5	883700-901					414
Guard Cartridges, 2/pk	9.4 x 15	7	820675-119	820675-119	820675-111	820675-111		165
Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-919	820950-901	820950-905	820950-908	820950-908	155
Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-919					155
Guard Hardware Kit	9.4 x 15		840140-901	840140-901	840140-901	840140-901		162
Guard Hardware Kit	4.6 x 12.5		820777-901	820777-901	820777-901	820777-901	820777-901	106
Guard Hardware Kit	2.1 x 12.5		820777-901	820777-901	820777-901	820777-901		106

Configurations not shown are available upon request.

High-Throughput HPLC Columns

ZORBAX CombiHT Columns
ZORBAX LC/MS Columns and Cartridge Columns
ZORBAX Rapid Resolution Columns

For . . .

- LC/MS Applications
- Combinatorial Chemistry Applications
- High Speed Chromatography
- Reduced Analysis Time

Agilent ZORBAX Columns are available in a variety of dimensions — from narrow-bore to preparative — to speed up your chromatography and increase your sample throughput, while still achieving as much resolution as you need.

Flow Rate Conversion

To determine the optimum flow rate for your 21.2 mm i.d. column, use the following equation:

$F_2 = F_1 \times (ID_2/ID_1)^2$

For example,

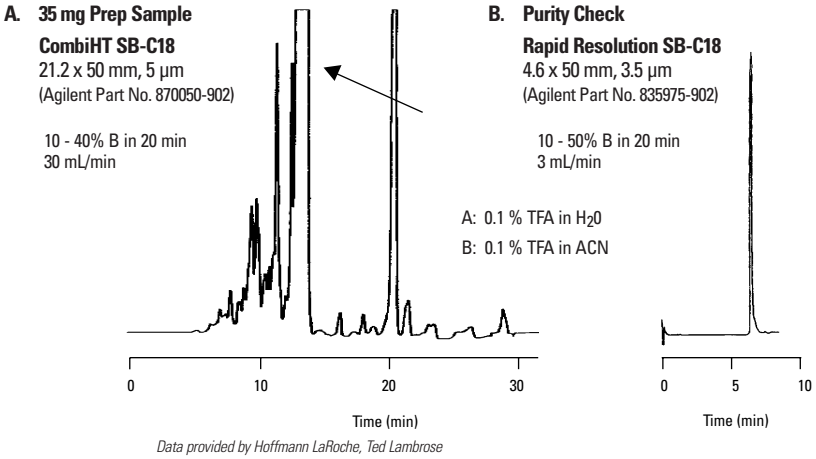
4.6 mm i.d.	21.2 mm i.d.
0.5 mL/min	10.5 mL/min
1.0 mL/min	21 mL/min
1.5 mL/min	31.5 mL/min

ZORBAX CombiHT HPLC Columns

- Fast preparative separations in the 15 - 50 mg range
- Three different column lengths — 50, 100, 150 mm
- Finger tight connections seal up to 7000 psi

CombiHT preparative columns are designed to scale up rapid analytical separations to sample loads of 15 - 50 mg. CombiHT columns use 5 µm packings for high efficiency and provide direct scale up from 50 - 150 mm analytical columns. These columns are available with reusable, finger tight endfittings so only the column barrel is replaced. CombiHT C8 and C18 columns are available in the two most popular ZORBAX bonded-phase types to optimize selectivity, StableBond for low pH and Eclipse XDB for mid-pH but can be packed with any ZORBAX phase on request.

FIGURE 1
Gradient Preparative High Throughput HPLC



Data provided by Hoffmann LaRoche, Ted Lambrose

The CombiHT column is used to separate and isolate a compound of interest, while the matching analytical column confirms the peak was isolated with high purity.

High-Throughput HPLC Columns

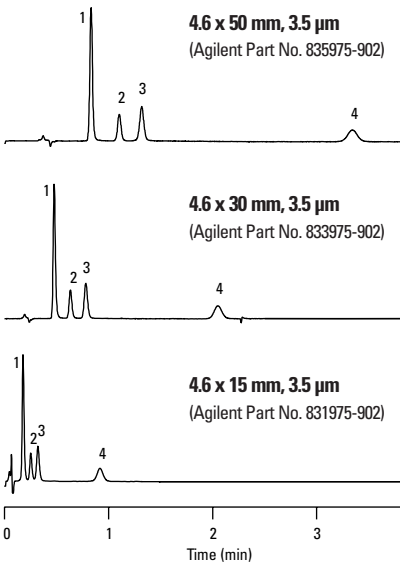
ZORBAX LC/MS HPLC Columns and Cartridge Columns

- Fast analysis for high speed LC/MS with Rapid Resolution cartridges
- Narrow bore and MicroBore columns for maximum sensitivity with Electrospray Ionization (ESI)
- Additional column dimensions for higher sample loads with APCI
- Many bonded phases available

Selecting a column for LC/MS involves three simple choices: column internal diameter, column length and bonded phase. ZORBAX, Rapid Resolution ultra-short, narrow-bore cartridge columns (2.1 x 15 and 2.1 x 30 mm) are often a first choice for LC/MS applications because these columns provide the fastest analysis time and good sensitivity with ESI detection. ZORBAX short, narrow-bore (2.1 x 50 mm) and MicroBore (1.0 x 50 mm) columns are also good choices for ESI/MS because their optimum flow rates are from 50 - 200 µL/min, providing additional sample resolution while still keeping analysis times very short. Even longer lengths are available for very complex samples. The 4.6 mm i.d. 15 and 30 mm cartridges as well as the longer columns provide the same resolution choices and are an ideal choice for APCI detectors where the low flow rate is not required.

ZORBAX StableBond and Eclipse XDB-C18 and C8 bonded phases are available in all configurations and additional bonded phases can be packed on request. StableBond columns demonstrate superior low pH stability and are ideal for formic acid and TFA containing mobile phases. Eclipse XDB bonded phases are a great choice with acetic acid mobile phases or for quick compound screening over a wide pH range.

FIGURE 2
Reduce Analysis Time By 70% Using Short LC/MS Cartridge Columns

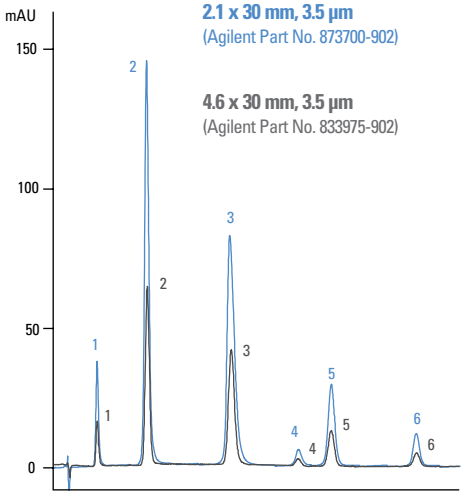


Column: **ZORBAX StableBond SB-C18**
Mobile Phase: 85% Water with 0.1% TFA
15% Acetonitrile
Flow Rate: 1.0 mL/min
Temperature: 35°C

Sample:
1. Phenylalanine
2. 5-Benzyl-3,6-dioxo-2-piperazine acetic acid
3. Asp-phe
4. Aspartame

To order or for technical assistance, call Agilent Technologies at 800-227-9770, or contact your local authorized Agilent distributor.

FIGURE 3
Maximize Sensitivity with Narrow-Bore LC/MS Cartridges



Column: **Rapid Resolution SB-C18, 3.5 µm**
Mobile Phase: Gradient: 5 - 35% B in 6 min
A: Water with 0.2% formic acid
B: Methanol with 0.2% formic acid
Temperature: 35°C

Sample: 2 µL Organic Acids
1. Gallic
2. Protocatechuic
3. Hydrocaffeic
4. Gentisic
5. Vanillic
6. Syringic

High-Throughput HPLC Columns

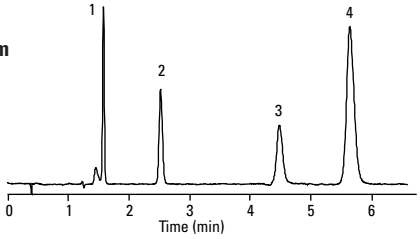
ZORBAX Rapid Resolution 3.5 µm HPLC Columns

- Reduce analysis time and solvent usage and increase sample throughput
- High efficiency in short and ultra-short column lengths
- Available in analytical (4.6 mm) and narrow-bore (2.1 mm) i.d.
- Comparable lifetime to 5 µm columns

FIGURE 4
Rapid Resolution Columns Reduce Analysis Time While Maintaining Resolution

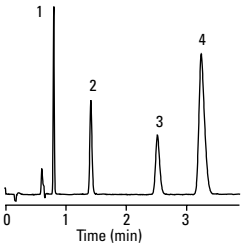
Bonus-RP, 4.6 x 150 mm, 5 µm
(Agilent Part No. 883668-901)

Flow Rate
1.5 mL/min



Rapid Resolution Bonus-RP
4.6 x 75 mm, 3.5 µm
(Agilent Part No. 866668-901)

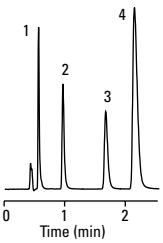
Flow Rate
1.5 mL/min



Column: **ZORBAX Bonus-RP**
Mobile Phase: 80% 25 mM NH_4Ac, pH 5.5
20% ACN
Temperature: 40°C
Detection: 254 nm
Sample:
1. Caffeine
2. Doxylamine
3. Chlorpheniramine
4. Triprolidine

Narrow Bore
Rapid Resolution Bonus-RP
2.1 x 50 mm, 3.5 µm
(Agilent Part No. 861700-901)

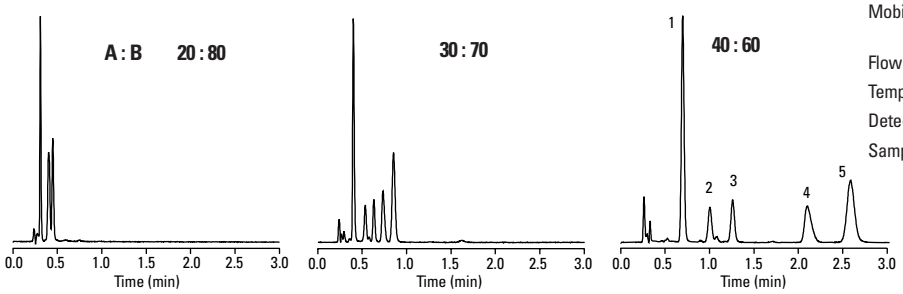
Flow Rate
0.3 mL/min



Rapid Resolution 3.5 µm columns are available in narrow bore configurations for great compatibility with LC/MS.

Agilent ZORBAX Rapid Resolution columns, with a 3.5 µm particle size, reduce analysis time and increase sample throughput for any application when compared to 5 µm columns. Rapid Resolution columns are available from 15 - 150 mm, in both 4.6 and 2.1 mm internal diameters, so the best configuration is available for high-throughput, LC/MS, combinatorial chemistry and rapid analytical applications. ZORBAX Rapid Resolution particles have superior mechanical strength, so every Rapid Resolution column has an extremely stable packed bed and provides a comparable lifetime to 5 µm columns. ZORBAX Rapid Resolution columns are available in all of the modern ZORBAX bonded phases — StableBond, Eclipse XDB, Bonus-RP and Extend-C18.

FIGURE 5
Run Method Development Chromatograms on Rapid Resolution Columns



Column: **ZORBAX StableBond SB-C18**
4.6 x 75 mm, 3.5 µm
(Agilent Part No. 866953-902)
Mobile Phase: A: 25 mM NaH_2PO_4, pH 3.0
B: MeOH
Flow Rate: 2.0 mL/min
Temperature: 35°C
Detection: UV 254 nm
Sample:
1. Diltiazem
2. Dipyridamole
3. Nifedipine
4. Lidoflazine
5. Flunarizine

Rapid Resolution 4.6 x 75 mm, 3.5 µm columns are a good choice for initial method development because they allow you to quickly determine if a given column and mobile phase combination can produce the desired separation.

ZORBAX High-Throughput Column Ordering Information

ZORBAX CombiHT Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7	Price
CombiHT	21.2 x 150	5	870150-902	870150-906	970150-902	970150-906	$ 1665
CombiHT	21.2 x 100	5	870100-902	870100-906	970100-902	970100-906	1248
CombiHT	21.2 x 50	5	870050-902	870050-906	970050-902	970050-906	827
CombiHT End Fittings (required for use)			820400-901	820400-901	820400-901	820400-901	203

ZORBAX Rapid Resolution LC/MS Cartridge Columns Ordering Information (requires Hardware Kit 820222-901)

Column Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7	Price
Rapid Resolution Cartridge	4.6 x 30	3.5	833975-902	833975-906	933975-902	933975-906	$ 194
Rapid Resolution Cartridge, 3/pk	4.6 x 30	3.5	833975-932	833975-936	933975-932	933975-936	547
Rapid Resolution Cartridge	4.6 x 15	3.5	831975-902	831975-906	931975-902	931975-906	155
Rapid Resolution Cartridge, 3/pk	4.6 x 15	3.5	831975-932	831975-936	931975-932	931975-936	436
Rapid Resolution Cartridge	2.1 x 30	3.5	873700-902	873700-906	973700-902	973700-906	194
Rapid Resolution Cartridge, 3/pk	2.1 x 30	3.5	873700-932	873700-936	973700-932	973700-936	547
Rapid Resolution Cartridge	2.1 x 15	3.5	875700-902	875700-906	975700-902	975700-906	155
Rapid Resolution Cartridge, 3/pk	2.1 x 15	3.5	875700-932	875700-936	975700-932	975700-936	436
Column Hardware Kit			820222-901	820222-901	820222-901	820222-901	162

ZORBAX Rapid Resolution and LC/MS Columns Ordering Information

ZORBAX StableBond Columns

Column Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-CN USP L10	SB-C3	SB-Phenyl USP L11	SB-Aq	Price
Rapid Resolution	4.6 x 150	3.5	863953-902	863953-906	863953-905		863953-912		$ 469
Rapid Resolution	4.6 x 75	3.5	866953-902	866953-906	866953-905		866953-912		414
Rapid Resolution	4.6 x 50	3.5	835975-902	835975-906	835975-905		835975-912		359
Solvent Saver Plus	3.0 x 150	3.5	863954-302	863954-306	863954-305		863954-312		469
Solvent Saver Plus	3.0 x 100	3.5		841954-306					435
Narrow Bore	2.1 x 150	5	883700-922	883700-906	883700-905	883700-909	883700-912		414
Narrow Bore	2.1 x 50	5	860975-902	860975-906	860975-905	860975-909	860975-912	860975-914	304
Narrow Bore RR*	2.1 x 150	3.5	830990-902	830990-906					469
Narrow Bore RR	2.1 x 50	3.5	871700-902	871700-906					359
MicroBore RR	1.0 x 150	3.5	863600-902	863600-906					505
MicroBore RR	1.0 x 50	3.5	865600-902	865600-906					388
MicroBore RR	1.0 x 30	3.5	861600-902	861600-906					301

ZORBAX Rx, Eclipse XDB and Bonus-RP Columns

Column Description	Size (mm)	Particle Size (µm)	Rx-C18	Rx-C8	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7	Eclipse XDB-Phenyl USP L11	Bonus-RP	Price
Rapid Resolution	4.6 x 150	3.5	863967-902	863953-906	963967-902	963967-906	963967-912	863668-901	$ 469
Rapid Resolution	4.6 x 75	3.5	866967-902	866953-906	966967-902	966967-906	966967-912	866668-901	414
Rapid Resolution	4.6 x 50	3.5			935967-902	935967-906	935967-912		359
Solvent Saver Plus	3.0 x 150	3.5			963954-302	963954-306	963954-312		469
Narrow Bore	2.1 x 150	5	883700-902	883700-906	993700-902	993700-906	993700-912	883725-901	414
Narrow Bore	2.1 x 50	5			960967-902	960967-906	960967-912	861971-901	304
Rapid Resolution	2.1 x 150	3.5			930990-902	930990-906			469
Rapid Resolution	2.1 x 50	3.5			971700-902	971700-906		861700-901	359
MicroBore RR*	1.0 x 150	3.5			963600-902	963600-906		863608-901	505
MicroBore RR	1.0 x 50	3.5			965600-902	965600-906		865608-901	388
MicroBore RR	1.0 x 30	3.5			961600-902	961600-906		861608-901	301

ZORBAX Extend-C18 Columns

Column Description	Size (mm)	Particle Size (µm)	Part Number	Price
Rapid Resolution	4.6 x 150	3.5	763953-902	$ 551
Rapid Resolution	4.6 x 75	3.5	766953-902	490
Rapid Resolution	4.6 x 50	3.5	735953-902	423
Narrow Bore	2.1 x 150	5	773700-902	490
Narrow Bore	2.1 x 50	5	760450-902	357
Narrow Bore RR*	2.1 x 50	3.5	735700-902	423
MicroBore RR	1.0 x 150	3.5	763600-902	505
MicroBore RR	1.0 x 50	3.5	765600-902	388
MicroBore RR	1.0 x 30	3.5	761600-902	301

*RR: Rapid Resolution 3.5 µm columns.

To order or for technical assistance, call Agilent Technologies at 800-227-9770, or contact your local authorized Agilent distributor.

Configurations not shown are available upon request. For more information on these and other columns consult the Agilent web site at **www.agilent.com**.

Agilent MicroBore and Capillary Columns

- High sensitivity for smallest sample sizes
- Compatibility with several LC/MS interfaces
- Several internal diameters — 1.0 mm, 0.5 mm, 0.3 mm
- Variety of bonded phases

MicroBore (1 mm) and capillary columns (0.5, 0.3 mm) are often the best choice when sample sizes are limited. MicroBore columns will improve limits of detection 5X over 2.1 mm i.d. columns, and capillary columns can decrease detection limits 20 – 50X relative to 2.1 mm i.d. columns when the same sample size is used. This increase in sensitivity can be critical. MicroBore and capillary columns use very low flow rates — from 1 – 50 µL/min. Therefore, these columns are ideal for use with detectors requiring the lowest flow rates, such as the particle beam and electrospray LC/MS detectors.

Agilent MicroBore and capillary columns are available in three different internal diameters and several lengths, so the ideal configuration is available for your sample limited applications. The 1.0 mm MicroBore columns are packed in stainless steel, and the 0.3 and 0.5 mm capillary columns are packed into glass-lined, stainless steel columns. These columns perform optimally with HPLC systems purchased or adjusted for microbore and capillary column use. Many bonded phases are available, including StableBond SB-C18 and SB-C8, Eclipse XDB-C18 and XDB-C8, Bonus-RP, Extend-C18, 300SB-C18 and Hypersil ODS.

ZORBAX MicroBore HPLC Column Ordering Information

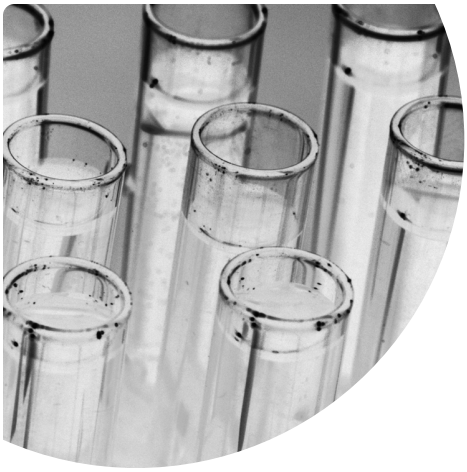
Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	300SB-C18	300SB-C8	Eclipse XDB-C18 USP L7	Eclipse XDB-C8 USP L7	Bonus-RP	Extend-C18	Price
1.0 x 150	3.5	863600-902	863600-906	863630-902	863630-906	963600-902	963600-906	863608-901	763600-902	$ 505
1.0 x 50	3.5	865600-902	865600-906	865630-902	865630-906	965600-902	965600-906	865608-901	765600-902	388
1.0 x 30	3.5	861600-902	861600-906			961600-902	961600-906	861608-901	761600-902	301

Agilent HPLC Capillary Columns Ordering Information (glass-lined)

Column	Size (mm)	Particle Size (µm)	SB-C18	300SB-C18	Eclipse XDB-C18	Price	Hypersil ODS	Price
Capillary	0.5 x 250	5	5064-8258	5064-8266	5064-8286	$ 505	5064-8278	$ 495
Capillary	0.5 x 150	5	5064-8256	5064-8264	5064-8287	455	5064-8289	446
Guard	0.5 x 35	5	5064-8254	5064-8294	5064-8296	301	5064-8282	295
Capillary RR*	0.5 x 150	3.5	5064-8262	5064-8268	5064-8288	505	5064-8275**	495
Capillary RR	0.5 x 35	3.5	5064-8260		5064-8298	356	5064-8299**	349
Capillary	0.3 x 250	5	5064-8257	5064-8265	5064-8269	505	5064-8276	495
Capillary	0.3 x 150	5	5064-8255	5064-8263	5064-8291	455	5064-8290	446
Guard	0.3 x 35	5	5064-8253	5064-8295	5064-8297	301	5064-8279	295
Capillary RR	0.3 x 150	3.5	5064-8261	5064-8267	5064-8271	505	5064-8277**	495

*RR: Rapid Resolution 3.5 µm columns

** 3 µm particles



ZORBAX Columns for BioAnalytical Chemistry

ZORBAX Wide-Pore 300StableBond HPLC Columns

- Four different bonded phases for reversed-phase selectivity optimization of peptides and proteins
- Exceptional stability and long lifetime at low pH with TFA containing mobile phases
- Excellent reproducibility
- Stable at high temperature — up to 80 - 90°C

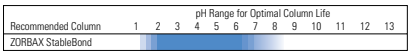
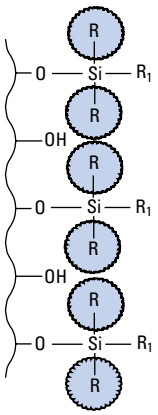


FIGURE 1
Sterically Protected 300StableBond Bonded Phase

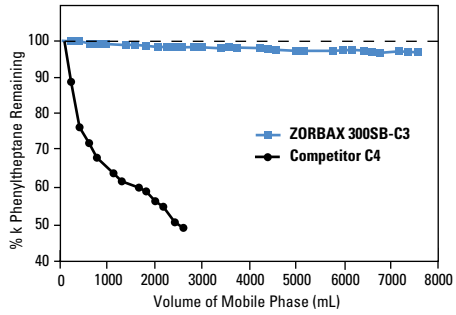


Bulky diisobutyl (C18) or diisopropyl (C8, C3, CN,) side-chain groups are used to stabilize both long and short chain bonded phases. Additional stability information on StableBond columns can be found on pages 8 -9.

ZORBAX 300StableBond columns are an ideal choice for the reproducible separations of proteins and peptides for two key reasons. First, wide-pore, 300Å columns are necessary for an efficient separation of proteins and peptides, or other large molecules, in order to allow these analytes to completely access the bonded phase. Second, 300StableBond columns are unmatched in their durability at low pH, such as with the TFA containing mobile phases typically used for protein and peptide separations. For LC/MS separations at low pH, 300StableBond columns can also be used with formic acid and acetic acid mobile phase modifiers. 300StableBond columns are available in four different bonded phases (C18, C8, C3, and CN) for selectivity and recovery optimization of proteins and polypeptides. To further increase sample recovery and improve efficiency for difficult proteins, 300StableBond columns can be used up to 80 - 90°C.

FIGURE 2
Short-Chain ZORBAX 300SB-C3 is Stable at Low pH, High Temperature

300StableBond columns are unsurpassed in their low pH stability. The reproducible monolayer bonding and sterically protected bonded phase increase the lifetime and reliability of the short-chain 300SB-C3 column over another wide-pore C4 column. Additional stability information on StableBond columns can be found on pages 8 -9.



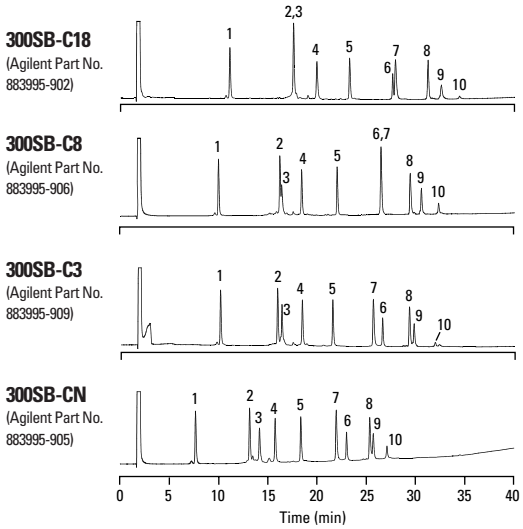
Mobile Phase: Gradients 0-100% B in 80 min.
A:0.5% TFA in Water
B:0.5% TFA in Acetonitrile
Isocratic Retention Test Conditions:
1-phenylheptane 50% A, 50% B
Columns: 4.6 x 150 mm
Temperature: 60°C
Flow Rate: 1.0 mL/min

ZORBAX StableBond 300SB Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits*	pH Range*	Endcapped	Carbon Load
ZORBAX 300SB-C18	300Å	45 m^2/g	90°C	1.0 - 8.0	No	2.8%
ZORBAX 300SB-C8	300Å	45 m^2/g	80°C	1.0 - 8.0	No	1.5%
ZORBAX 300SB-C3	300Å	45 m^2/g	80°C	1.0 - 8.0	No	1.1%
ZORBAX 300SB-CN	300Å	45 m^2/g	80°C	1.0 - 8.0	No	1.2%

*300StableBond columns are designed for optimal use at low pH. At pH 6-8, highest column stability for all silica-based columns is obtained by operating at temperatures <40°C and using low buffer concentrations in the range of 0.01-0.02M. At mid or high pH, 300Extend-C18 is recommended.

FIGURE 3
Four Different 300SB Bonded Phases Optimize Separation of Large Polypeptides



Columns: **StableBond 300SB** 4.6 x 150 mm, 5 µm
Mobile Phase: Linear Gradient, 25 - 70% B in 40 min.
A: 0.1% TFA in Water
B: 0.09% TFA in 80% Acetonitrile/20% Water
Flow Rate: 1.0 mL/min
Temperature: 60°C
Sample: 3 µg each protein

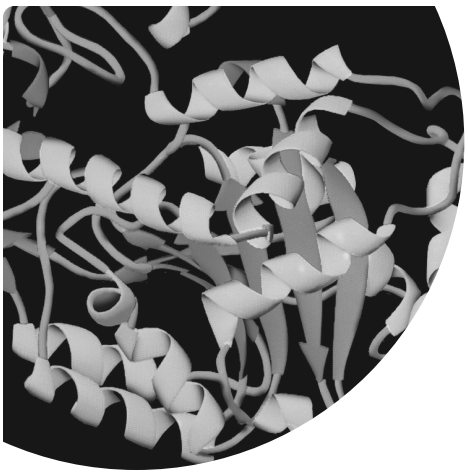
1. RNase
2. Insulin
3. Cytochrome C
4. Lysozyme
5. Parvalbumin
6. CDR
7. Myoglobin
8. Carbonic Anhydrase
9. S-100β
10. S-100α

The 300SB-C18, C8, C3 and CN bonded phases all provide a different separation of this group of polypeptides. This adds an important parameter for quickly optimizing protein separations. The 300SB-CN column offers unique selectivity for more hydrophilic polypeptides.

ZORBAX StableBond (300Å) Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3	Price
Standard Columns and Bulk Packing							
Preparative	21.2 x 250	7	880995-102	880995-106	880995-109	880995-105	$ 2318
Semipreparative	9.4 x 250	5	880995-202	880995-206	880995-209	880995-205	1099
Analytical	4.6 x 250	5	880995-902	880995-906	880995-905	880995-909	469
Analytical	4.6 x 150	5	883995-902	883995-906	883995-905	883995-909	414
Analytical	4.6 x 50	5	860950-902	860950-906	860950-905	860950-909	325
Rapid Resolution	4.6 x 150	3.5	863973-902	863973-906	863973-905	863973-909	469
Rapid Resolution	4.6 x 50	3.5	865973-902	865973-906	865973-905	865973-909	359
Solvent Saver Plus	3.0 x 100	3.5		884950-565			435
Narrow Bore	2.1 x 150	5	883750-902	883750-906	883750-905	883750-909	414
Narrow Bore RR*	2.1 x 150	3.5		863750-906			469
Narrow Bore RR	2.1 x 50	3.5	865750-902	865750-906			359
MicroBore RR	1.0 x 150	3.5	863630-902	863630-906			505
MicroBore RR	1.0 x 50	3.5	865630-902	865630-906			388
Bulk Packing, 2 grams		5		820966-306			136
Guard Cartridges, 2/pk	9.4 x 15	7	820675-124	820675-124	820675-124	820675-124	165
Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-921	820950-918	820950-923	820950-924	155
Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-918	821125-918	821125-924	821125-924	155
Guard Hardware Kit	9.4 x 15		840140-901	840140-901	840140-901	840140-901	162
Guard Hardware Kit	4.6 x 12.5		820777-901	820777-901	820777-901	820777-901	106
Guard Hardware Kit	2.1 x 12.5		820777-901	820777-901	820777-901	820777-901	106
Capillary Glass-lined Columns							
Capillary	0.5 x 250	5	5064-8266				505
Capillary	0.5 x 150	5	5064-8264				455
Capillary RR	0.5 x 150	3.5	5064-8268				505
Capillary	0.3 x 250	5	5064-8265				505
Capillary	0.3 x 150	5	5064-8263				455
Capillary RR	0.3 x 150	3.5	5064-8267				505
Guard Cartridges	0.5 x 35	5	5064-8294				301
Guard Cartridges	0.3 x 35	5	5064-8295				301

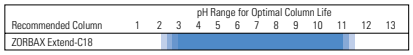
Configurations not shown are available upon request.
*RR: Rapid Resolution 3.5 µm columns.
StableBond (80Å) columns are also available for small molecule separations.
See page 10 for descriptions, ordering information and additional information on StableBond columns.



ZORBAX Columns for BioAnalytical Chemistry

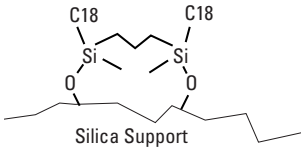
ZORBAX 300Extend-C18 HPLC Columns

- Rugged, high and low pH separations of polypeptides and peptides from pH 2 - 11.5
- Different selectivity possible at high and low pH
- High efficiency and good recovery of hydrophobic peptides at high pH
- Ideal for LC/MS with ammonium hydroxide modified mobile phase



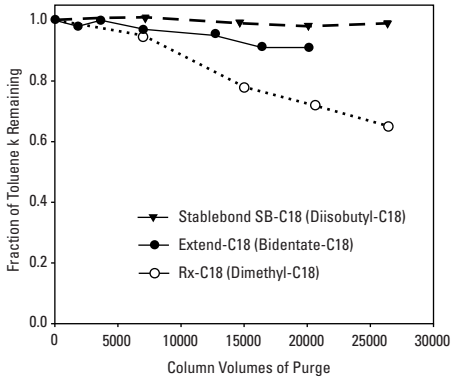
ZORBAX 300Extend-C18 is a wide-pore HPLC column for the high efficiency separations of peptides from pH 2 - 11.5. The unique bidentate bonded phase provides excellent lifetime and reproducibility at high and low pH. At high pH, retention and selectivity of peptides and polypeptides can change dramatically as a result of changes in charge on molecules. Excellent recoveries of hydrophobic polypeptides have been achieved at room temperature and high pH. LC/MS sensitivity of peptides and polypeptides can also be improved at high pH using a simple ammonium hydroxide containing mobile phase.

FIGURE 1
New Bidentate C18-C18 Bonding for Extend-C18 Bonded Phase



The combination of the unique bidentate structure and double endcapping permits long life at high pH.

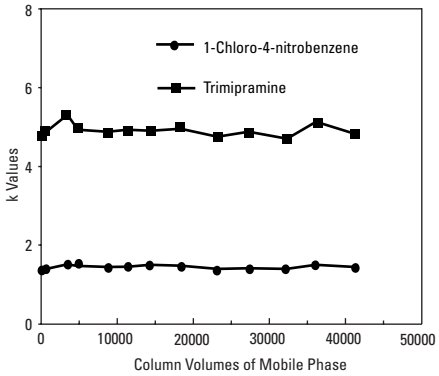
FIGURE 2
Extend-C18 and StableBond SB-C18 are Stable at Low pH



Columns: 4.6 x 150 mm, 5 µm
Column Aging: 50% Methanol : 50% Water + 1% TFA
Flow Rate: 1.5 mL/min, continuous
Temperature: 90°C
Column Test: 60% Methanol : 40% Water
Flow Rate: 1.5 mL/min
Temperature: RT
Test Solute: Toluene

The 300Extend-C18 column can be used at high and low pH — from pH 2 - 11.5. This chart shows that the 300Extend-C18 has the needed stability at low pH for long-term reproducible separations. Therefore one wide-pore column can be used for selectivity optimization at low and high pH with both TFA and ammonium hydroxide mobile phases. Additional information on Extend-C18 columns can be found on pages 18 - 19.

FIGURE 3
Long Life at High pH with Extend-C18



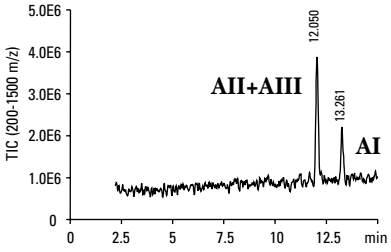
Column: **ZORBAX Extend-C18**, 4.6 x 150 mm
Mobile Phase: 20% 20 mM NH_4OH, pH 10.5
80% Methanol
Flow Rate: 1.5 mL/min
Aging at 24°C, tests at 40°C

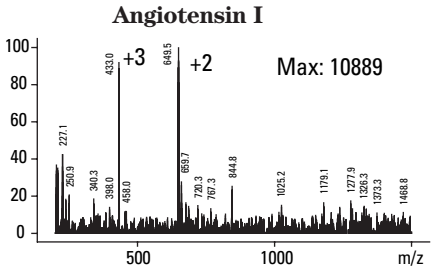
Each 10,000 column volumes is approximately one working month.

To order or for technical assistance, call Agilent Technologies at 800-227-9770, or contact your local authorized Agilent distributor.

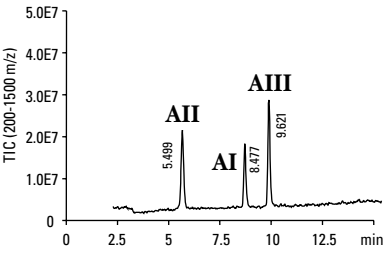
FIGURE 4
LC/MS Analysis of Angiotensin on Extend-C18

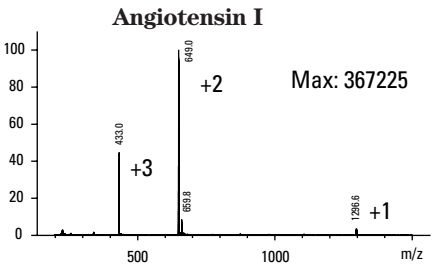
A. Acidic Conditions: A: 0.1% TFA in water B: 0.085% TFA in 80% acetonitrile (ACN)





B. Basic Conditions: A: 10 mM NH_4OH in water B: 10 mM NH_4OH in 80% ACN



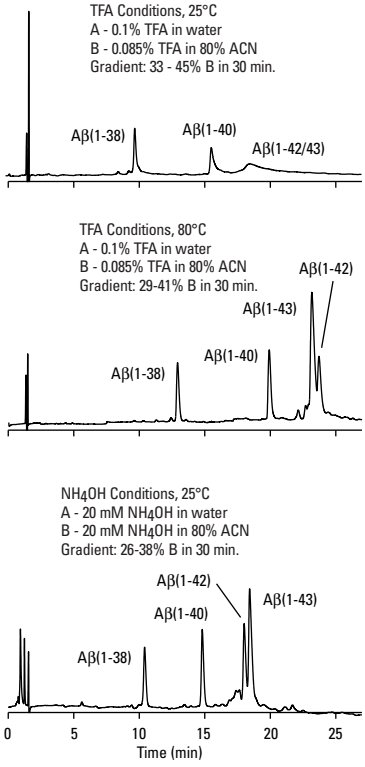


Column: **ZORBAX Extend-C18**
2.1 x 150 mm, 5 µm
(Agilent Part No. 773700-902)
Flow Rate: 0.2 mL/min
Temperature: 35°C
Mobile Phase: As indicated
Gradient: 15-50% B in 15 min.
LC/MS: Pos. Ion ESI- Vf 70V, Vcap 4.5 kV, N2- 35 psi, 12 L/min., 325°C
Sample: Angiotensin I, II, III
2.5 µL sample (50 pmol each)
Angiotensin I: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu
Angiotensin II: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
Angiotensin III: Arg-Val-Tyr-Ile-His-Pro-Phe

Reference: Boyes, B.E., *Separation and Analysis of Peptides at High pH Using RP-HPLC/ESI-MS*, 4th WCBP, San Francisco, CA Jan 2000.

Both small and large peptides demonstrate selectivity changes at high and low pH. At high pH, due to a change in charge, all three Angiotensins can be resolved. In addition, the spectral clarity of Angiotensin I is dramatically improved at high pH with the ammonium hydroxide mobile phase. The Extend-C18 column can be used for the analysis of small peptides at high pH as well (see pages 18 - 19 for additional details).

FIGURE 3
High pH Can be Used for Separating Hydrophobic or Other Low Solubility Peptides



Column: **ZORBAX 300Extend-C18**, 2.1 x 150 mm, 3.5 µm
(Agilent Part No. 763750-902)
Flow Rate: 0.25 mL/min

At high pH and room temperature the hydrophobic amyloid peptides show better recovery and a different selectivity than at low pH. At low pH elevated temperature is necessary to achieve the same recovery as at high pH.

ZORBAX 300Extend-C18 Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX 300Extend-C18	300Å	45 m^2/g	60°C*	2.0 - 11.5	Double	4%

**Temperature limits are 60°C up to pH 8, 40°C for pH 8 - 11.5.*

ZORBAX 300Extend-C18 Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	Part Number	Price
Analytical	4.6 x 250	5	770995-902	$ 551
Analytical	4.6 x 150	5	773995-902	490
Rapid Resolution	4.6 x 150	3.5	763973-902	551
Rapid Resolution	4.6 x 50	3.5	765973-902	423
Narrow Bore	2.1 x 150	3.5	763750-902	551
Narrow Bore	2.1 x 50	3.5	765750-902	423
Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-932	184
Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-932	184
Guard Hardware Kit			820777-901	106

Configurations not shown are available upon request.

Extend-C18 (80Å) columns are also available. See page 18 for descriptions, ordering information and additional information on Extend-C18 columns.

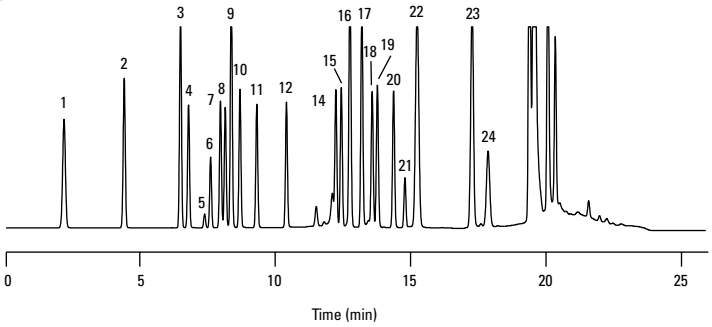
ZORBAX Columns for BioAnalytical Chemistry

ZORBAX Eclipse AAA (Amino Acid Analysis) HPLC Columns

- High resolution and rapid analysis of 24 amino acids
- Use tested for Amino Acid Analysis
- Uses well known OPA and FMOC derivatization chemistry
- Easily automated using a detailed on-line, derivatization protocol available for use with Agilent 1100 Autosampler

The new ZORBAX Eclipse AAA high efficiency HPLC column rapidly separates amino acids following an updated and improved protocol. Total analysis from injection to injection can be achieved in as little as 14 min (9 min. analysis time) on the short, 7.5 cm length column and 24 min (18 min. analysis time) on the 15 cm column length. Exceptional sensitivity (5 – 50 pmol with DAD, FLD) and reliability are achieved using both OPA and FMOC derivatization chemistries in one fully automated procedure using the Agilent 1100 HPLC instrument.

FIGURE 1
High Resolution of 24 Amino Acids Using ZORBAX Eclipse-AAA Protocol



Column:	**ZORBAX Eclipse AAA** 4.6 x 150 mm, 3.5 µm (Agilent Part No. 963400-902)
Mobile Phase:	A: 40 mM Na_2HPO_4, pH 7.8 B: ACN : MeOH : water, 45:45:10 v/v
Flow Rate:	2.0 mL/min
Temperature:	40°C
Detector:	Fluorescence

1. Asp	9. Cit	17. Trp
2. Glu	10. Arg	18. Phe
3. Asn	11. Ala	19. Ile
4. Ser	12. Tyr	20. Leu
5. Gln	13. Cys	21. Lys
6. His	14. Val	22. Hyp
7. Gly	15. Met	23. Sar
8. Thr	16. Nva	24. Pro

This high resolution separation of 24 amino acids is done in 18 minutes. If the Rapid Resolution 4.6 x 75 mm Eclipse AAA column is selected these amino acids are resolved in 9 minutes.

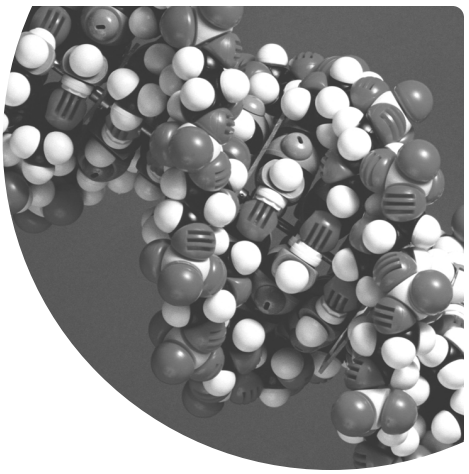
ZORBAX Eclipse AAA Ordering Information

Column Description	Size (mm)	Particle Size (µm)	Part Number	Price
Analytical routine sensitivity	4.6 x 150	5	993400-902	$ 414
Analytical high sensitivity, high-resolution work using the FLD	4.6 x 150	3.5	963400-902	469
Analytical routine sensitivity, high-throughput	4.6 x 75	3.5	966400-902	414
Solvent Saver high sensitivity, high-resolution	3.0 x 150	3.5	961400-302	469
Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-931	155
Guard Hardware Kit			820777-901	106

For a detailed description of the Eclipse AAA protocol using the Agilent 1100 HPLC request publication number 5980-1193E or download it from the Agilent website at **www.agilent.com**.

ZORBAX Columns for BioAnalytical Chemistry

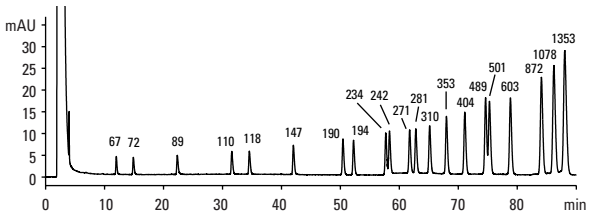
ZORBAX Eclipse dsDNA Analysis HPLC Columns



- High sensitivity and resolution for dsDNA fragments
- Separate from 20 - 1500 base pairs
- 20-50X the capacity of non-porous columns
- Usable for DHPLC, PCR fragment analysis and other dsDNA sizing applications

The ZORBAX Eclipse dsDNA analysis column provides high-resolution, automated, quantitative separations of dsDNA fragments by HPLC. Up to 1 bp resolution can be achieved depending on fragment length and gradient analysis time. For high sensitivity, load up to 10 µg and detect less than 1 ng/band using the 2.1 mm i.d. columns. Or, inject up to 40 µg on the 4.6 mm i.d. columns for higher capacity quantitative separations. This column is useful for applications such as PCR fragment separations, restriction digest separations, DHPLC, RFLP and RAPD.

FIGURE 1
High Resolution Separation on a ZORBAX Eclipse dsDNA Analysis Column



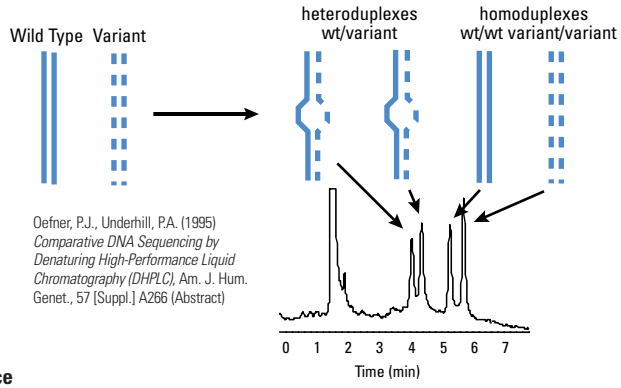
Column:	**Eclipse dsDNA**, 2.1 x 150 mm, 3.5 µm	Detection:	UV 260 nm
Mobile Phase:	Gradient: 45 – 75% B in 90 minutes A: 0.1 M TEAA*, 0.1 mM EDTA, pH 7.0 B: A + 25% ACN	Sample:	200 ng restriction digest standards
		Instrument:	Agilent 1100, 1 µL flow cell, binary pump
Flow Rate:	0.2 mL/min		
Temperature:	50°C		

*TEAA = triethylammonium acetate

FIGURE 2
Mutation Detection by Denaturing HPLC (DHPLC)

DHPLC analysis of the Y chromosome STS, DYS271 shows ideal separation

The Eclipse dsDNA Analysis column can be used for denaturing HPLC — DHPLC — and several other special techniques. In this example a DHPLC analysis of the Y chromosome STS, DYS271 shows ideal separation of the homo- and heteroduplexes formed.

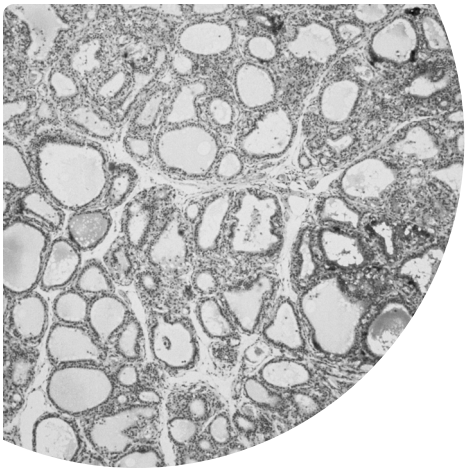


Oefner, P.J., Underhill, P.A. (1995) *Comparative DNA Sequencing by Denaturing High-Performance Liquid Chromatography (DHPLC),* Am. J. Hum. Genet., 57 [Suppl.] A266 (Abstract)

ZORBAX Eclipse dsDNA Ordering Information

Column Description	Size (mm)	Particle Size (µm)	Part Number	Price
Analytical	2.1 x 75	3.5	945750-902	$ 551
Analytical	2.1 x 150	3.5	993850-902	622
Analytical	3.0 x 75	3.5	945750-302	551
Preparative	4.6 x 75	3.5	992750-902	551
Guard Cartridge, 4/pk	2.1 x 12.5	3.5	821125-929	155
Guard Cartridge, 4/pk	4.6 x 12.5	3.5	820950-929	155
Guard Hardware Kit			820777-901	106

To order or for technical assistance, call Agilent Technologies at 800-227-9770, or contact your local authorized Agilent distributor.



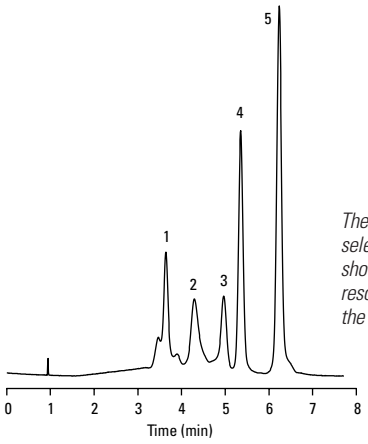
ZORBAX Columns for BioAnalytical Chemistry

ZORBAX GF-250/450 Size Exclusion HPLC Columns

- High efficiency and reproducibility with short analysis times
- Hydrophilic diol bonded phase for good protein recovery
- Compatible with organic modifiers and denaturants
- Wide usable pH range (pH 3 - 8)

ZORBAX GF-250 and GF-450 size exclusion columns are ideal for the size separations of proteins and other biomolecules. The separation range is 4,000 - 900,000 for globular proteins when using GF-250 and GF-450 columns in series. The GF-250/450 size exclusion columns have a hydrophilic diol bonded phase for high recovery of proteins (typically >90%) and a unique zirconia modification of the silica to extend the pH range from 3 - 8. The GF-250 and GF-450 columns are packed with precisely sized porous silica microspheres with narrow pore size and particle size distributions. The result is a highly efficient, rugged and reproducible size exclusion column for separations of proteins with flow rates of up to 3 mL/min. These columns are compatible with organic modifiers and denaturants in the mobile phase to eliminate protein aggregation for proper size determination. Some common applications include separations of protein monomers, dimers and aggregates, desalting, protein molecular weight estimation and separations of modified proteins.

FIGURE 1
Separation of Protein Standards on the ZORBAX GF-250 SEC Column



The protein standards separated here are a commonly selected set of standards. The ZORBAX GF-250 column shows excellent resolution for this sample. Additional resolution of the thyroglobulin can be obtained by adding the GF-450 column in series.

Column: **ZORBAX GF-250**, 9.4 x 250 mm
Mobile Phase: 200 mM Sodium Phosphate, pH 7.0
Flow Rate: 2 mL/min
Temperature: Ambient
Detection: UV 254 nm
Sample: BioRad Gel Filtration Standards for Size Exclusion

1. Thyroglobulin	670,000 Da
2. Bovine Gamma Globulin	158,000 Da
3. Chicken Ovalbumin	44,000 Da
4. Equine Myoglobin	17,000 Da
5. Vitamin B-12	1,350 Da

ZORBAX GF-250 and GF-450 Gel Filtration Columns Ordering Information

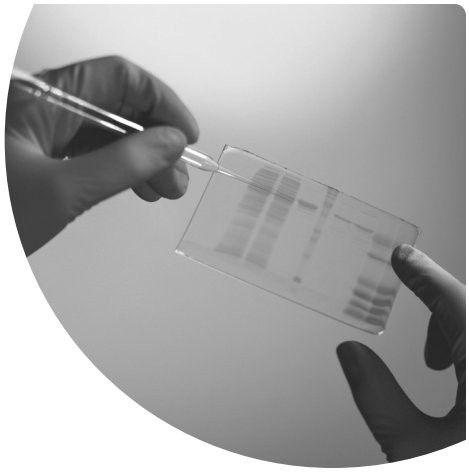
Column Description	Size (mm)	Particle Size (µm)	Part No.	Price
GF-250, 150Å pore size	21.2 x 250	4	884974-901	$ 2754
GF-250, 150Å pore size	9.4 x 250	4	884973-901	878
GF-250, 150Å pore size	4.6 x 250	4	884973-701	469
GF-450, 300Å pore size	21.2 x 250	6	884974-910	2754
GF-450, 300Å pore size	9.4 x 250	6	884973-902	878
GF-250 Guard Cartridge*, 2/pk	9.4 x 15	4	820675-111	165
GF-250 Guard Cartridge, 4/pk	4.6 x 12.5	4	820950-911	155
GF-450 Guard Cartridge, 2/pk	9.4 x 15	4	820675-111	165
GF-450 Guard Cartridge, 4/pk	4.6 x 12.5	4	820950-911	155
GF-250, GF-450 Hardware Kit	9.4 x 15		840140-901	162
GF-250, GF-450 Hardware Kit	4.6 x 12.5		820777-901	106

*Diol Phase

ZORBAX GF-250 and GF-450 Column Specifications

Bonded Phase	Pore Size	Particle Size	MW Range	Surface Area	pH Range	Flow Rate	Max Pressure
ZORBAX GF-250	150Å	4 µm	4,000 - 400,000	140 m^2/g	3.0 - 8.0	<3.0 mL/min	5000 psi
ZORBAX GF-450	300Å	6 µm	10,000 - 900,000	50 m^2/g	3.0 - 8.0	<3.0 mL/min	5000 psi

ZORBAX Columns for BioAnalytical Chemistry

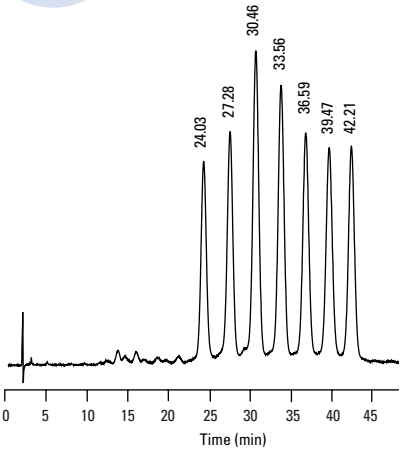


ZORBAX Oligo HPLC Columns

- Separate oligonucleotides up to 60 - 80 mer lengths
- High-resolution gradient analysis with mixed mode ion exchange and reversed phase column
- Isolate oligonucleotides with >95% purity
- Denaturatants can be added to the mobile phase

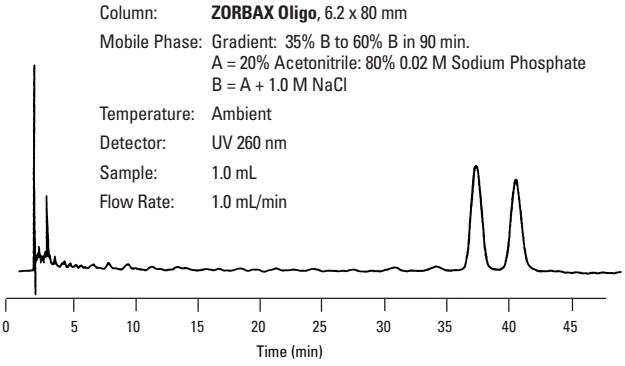
The ZORBAX Oligo column is designed for the high-resolution gradient separation and purification of oligonucleotides. Oligonucleotides having 3 - 48 bases have successfully been purified using this column, while it is possible to separate oligonucleotides up to the 60 - 80 mer range. The Oligo column uses a mixed mode ion exchange and reversed phase bonded phase developed specifically for oligonucleotides in order to effectively isolate them with high purity. Simple gradient mobile phases are used for oligomer separations with the possible addition of denaturants, such as urea, to prevent self-hybridization.

FIGURE 1
Separation of Standard p(DT) 12 - 18 on ZORBAX Oligo Column



Column: **ZORBAX Oligo**, 6.2 x 80 mm
Mobile Phase:
Gradient: 35 - 80% B in 90 min.
A:20:80 ACN: 20 mM Sodium Phosphate pH 7.0
B:20:80 ACN: 20 mM Sodium Phosphate pH 7.0 + 0.8 M NaCl
Flow: 1.0 mL/min
Temperature: Ambient
Detector: UV 260 nm
Sample Volume: 50 µL, 5 units/mL

FIGURE 2
Separation of 24 and 25-mer Oligonucleotides


Column: ZORBAX Oligo, 6.2 x 80 mm
Mobile Phase: Gradient: 35% B to 60% B in 90 min.
A = 20% Acetonitrile: 80% 0.02 M Sodium Phosphate
B = A + 1.0 M NaCl
Temperature: Ambient
Detector: UV 260 nm
Sample: 1.0 mL
Flow Rate: 1.0 mL/min
0 5 10 15 20 25 30 35 40 45
Time (min)

ZORBAX Bio Series Oligo Column Specifications

Bonded Phase	Pore Size	Surface Area	pH Range	Bonded Functionality
ZORBAX Oligo	150Å	140 m^2/g	2.5 – 8.5	Proprietary diol

ZORBAX Oligo Column Ordering Information

Size (mm)	Particle Size (µm)	Part Number	Price
6.2 x 80	5	820940-901	$ 469
9.4 x 250	5	884973-903	1214
21.2 x 250	5	884974-915	3256

Configurations not shown are available upon request.

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